PHARMACOGNOSTIC AND ANTI-MALARIAL STUDIES OF *BAPHIA PUBESCENS* HOOK F. (LEGUMINOSAE)

BY

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CERTIFICATION

I, **ANOWI Chinedu Fredrick,** a doctoral student of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka with Registration Number **201365A7002P,** hereby certify that the work embodied herein is original and has not been submitted in part or full for any other Diploma or Degree of this or any other University.

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APPROVAL

The title "Pharmacognostic and Anti-Malarial Studies of *Baphia* p*ubescens* Hook. F. (Leguminosae)" has been approved for **ANOWI Chinedu Fredrick**, a doctoral student with Registration Number **201365A7002P**, of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

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Dedicated with Humility and Reverence to Mary

Mother of Jesus the Saviour

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May the good Lord bless all of you.

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LIST OF ABBREVIATIONS

HPLC-DAD: High-performance liquid chromatography-diode array detection

ALT: Alanine Amino Transferase

AST: Aspartate Amino Transferase

ALP: Alkaline phosphatase

VLC: Vacuum liquid chromatography

TLC: Thin layer chromatography

WBC: White blood cell

RBC: Red blood cell

PCV: Pack cell volume

nm: Nanometer

mg: Milligram

Kg: Kilogram

min: Minutes

h: Hours

mL: Milliliter

rpm: Revolutions per minute

g: Gram

n-Hex: *n*-Hexane

EtOAc: Ethyl acetate

DCM: Dichloromethane

MeOH: Methanol

ABSTRACT

Despite the availability of drugs for malaria, the disease is still one of the most important infectious diseases of humans. With reports of decreased sensitivity to current first line drugs, there arises the need for newer drugs to combat this problem. This has led to increase in research in the investigation of a new alternative source for treatment of malaria including medicinal plant. This study is part of efforts and resources geared towards the malaria control in Nigeria, where the burden of malaria is greatest in Africa due to the large population. In this study, nine Nigerian medicinal plants: *Kigelia africana, Baphia pubescens, Morinda lucida, Synclisa scabrida and Buchholzia coriaceae*, *Rauwolfia vomitoria*, *Nauclea latifolia, Anthocleista djalonensis and Moringa oleifera* were preliminarily evaluated for antimalarial property. Crude methanol extracts of the plants were prepared by cold maceration of the dried and powdered leaves. Preliminary phytochemical screening and antimalarial assay of the plants were carried out using standard methods. The plant with the most effective antimalarial activity was selected, extracted and fractionated using *n*-hexane, ethyl acetate and *n*-butanol. The fractions were tested for their antimalarial property, and the most active fraction was subjected to vacuum liquid chromatography (VLC) using solvent mix with varying polarity. The resulting subfractions from the VLC were tested for their antimalarial property, and the most effective were subjected to size exclusion chromatography (Sephadex). These Sephadex subfractions were bulked up with the aid of TLC and UV light to get 19 subfractions which were subjected to HPLC-DAD analysis. In the preliminary studies, the nine plants tested showed varying degree of anti-malarial property. At 100 mg/kg each of them had the following percentage curative effect – *A. djalonensis* 54%, *M. oleifera* 58%, *S. scabrida* 78%, *B. coriaceae* 78%, *N. latifolia* 81%, *M. lucida* 85%, *K. africana* 90%, *R. vomitoria* 92%, and *B. pubescens* 97%. *B. pubescens* was selected for further investigations because of its promising antimalarial activity and safety at (LD_{50}) 5000/kg. Results of microscopic examination showed that the leaves contain trichomes, spiral xylem vessels and anomocytic stomata. The extractive values fall within normal range. The main phytochemical constituents detected in the ethyl acetate fraction include - tannins, carbohydrates, saponins, flavonoids, alkaloids, and glycosides. Results of the antimalarial screening of the *n*-hexane, ethyl acetate, and *n*-butanol fractions of *B. pubescens* showed that the ethyl acetate fraction was the most effective with almost 100% cure at 100 mg/kg. This ethyl acetate fraction was subjected to VLC and Sephadex separation techniques and some sub-fractions were obtained. These subfractions were subjected to antimalarial screening. HPLC-DAD analysis of the active subfractions of *B. pubescens* ethyl acetate fraction revealed the presence of citreodimene F, a sesquiterpene lactone. Since sesquiterpene lactone are generally known for their antimalarial properties, citreodimene F may be responsible for the antimalarial property of the plant. Also, the abundance of palitantin, an antiprotozoal agent with antileishmania properties, was revealed. This compound may confer an antileishmanial activity on the plant. Other biologically active compounds detected include aureonitol, cytosporin D, septicine, cerebroside, and shanzhiside methyl ester. The results of this work justify the empirical use of *B. pubescens* in traditional medicine for the treatment of malaria. It is hoped that the information and data present herein will be useful in rolling back this disease.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Malaria is a major health problem in Africa, Asia, Central and South America. Unless precautions are taken, anyone living in or traveling to a country where malaria is present can contract the disease (Tilley *et al.*, 2011). Malaria occurs in about 100 countries; approximately 40% of the world population is at risk of contracting malaria (Renia *et al*., 2012). The incubation period for malaria symptoms is about one to three weeks but may be extended to eight to 10 months after the initial infected mosquito bites occur (Abba *et al.*, 2011). Some people may have dormant parasites that they may get reactivated years after the initial infection.

Malaria is diagnosed by the patient's history of recurrent symptoms and the identification of the parasites in the blood (Kattenberg *et al.,* 2011). It is usually treated by using combinations of two or more anti-parasitic drugs. More serious infections are treated with intravenous anti-parasitic drugs in the hospital (Wilson, 2012). Infants, children, and pregnant females, along with immune suppressed patients are at higher risk for worse outcomes when infected with malaria parasites (Perkins and Bell 2008; Pates and Curts, 2005).

To reduce the chance of getting malaria, people should avoid malaria - endemic areas of the world, use mosquito repellents, cover exposed skin, and use mosquito netting covered areas when sleeping (Harper and Armelagos, 2011). The prognosis for the majority of malaria patients is good. Most recover with no problem unless infected with *P. falciparum* or *P. knowlesi*, which may have fair to poor outcomes unless treated immediately (Prugnolle *et al.,* 2011).

Malaria remains a major health problem in tropical countries in terms of geographical spread, high morbidity, high mortality, and severe complication – especially among children. Following some important initial results obtained in the sixties with an ambitious project of disease eradication, in the mid-seventies there was an alarming resurgence of the infection in places, particularly in South-East Asia, that had been partly or completely freed of it (Sallares, 2002). Many factors have contributed to the defeated earlier hopes of containing or abating the disease such as the poor socio-economic status of the involved countries, precluding adequate government funding, faulty planning and management, quantitative and qualitative inadequacy of available manpower, and the emergency and rapid spreading of resistance both of vector mosquitoes to insecticides and of pathogenic plasmodia to anti-malarial drug (Hays 2005; Bruce-Chwatt, 1980).

The world Health Organization has estimated that in 2010, there were 219 million documented cases of malaria. That year, the disease killed between 660, 000 to 1.2 million people (Nayyar *et al.,* 2012), many of whom were children in Africa. The actual number of deaths is not known with certainty, as accurate data is unavailable in many rural areas, and many cases were undocumented. Malaria is known as tazo or tazomoka in local terminology in Madagascar. Within the context of traditional practice, malaria (and/or malaria symptoms) is commonly treated with decoctions or infusions from bitter plants (Keating, 2012).

Malaria which was initially recognized as episodic fever was probably introduced into Madagascar from mainland Africa by immigration. It was reported as early as 1602 on the coast of Madagascar (Beare *et al.,* 2011) and has remained a public health problem (Randrianarivelojosia *et al.*, 2002). The Malagasy words *tazo* and *tazomoka* refer to the clinical signs suggestive of malaria: principally, fever, headache, backache, shivering and fatigue.

Malagasy people, particularly those living in the countryside, use traditional plants to fight infectious diseases (Ohiri *et al.*, 1989). The current use of plants for medicinal purpose reflects the attachment of the people to their culture and a lack of access to modern medicine (associated with poverty in most cases). The resistance of *Plasmodium* spp. to drugs – such as chloroquine – has become a serious problem in areas of endemic malaria and in malaria-free areas with occasional imported cases (Gratz, 2006).

1.2 STATEMENT OF PROBLEMS

There is an urgent need for new antimalarial agents that are highly effective and possess low toxicity. This search is driven by the development of resistance of the malarial parasite to existing compounds, as well as the toxicity of drugs used in the treatment of malaria. The discovery of novel therapeutic molecules from plants is an important alternative to overcome the increasing levels of antimalarial drug resistance and the declining number of potent, safe and nontoxic drugs available against the disease.

With the potentials possessed by plants, in drug discovery, this research seeks to investigate the antimalarial activites of Nigerian plants, and determine the bioactive secondary metabolites responsible for therapeutic potentials of the plants against malaria disease.

1.3 JUSTIFICATION OF STUDY

The search for new antimalarial drugs based on plants is important due to the emergence and widespread of chloroquine-resistant and multiple drug-resistant malaria parasites, which require the development of new antimalarials. The continuous evolution of microbial resistance to the currently available anti-microbial has necessitated the search for novel and effective

antimicrobial compounds to which these microbes are yet to develop resistance (Farnsworth, 1982). With the rising problems of side effects, cost and limited efficacy of anti-microbial drugs, there is an urgent and compelling need for the development of alternative antimicrobial substances. Also, researchers are nowadays turning to natural products from plants (Kumar *et al*., 2008), as their main source of bioactive compounds with antimicrobial properties to complement the existing synthetic antimicrobials that are gradually becoming less potent against pathogenic microorganisms. Hence, there is the need for the phytochemical screening and investigations into the antimalarial potentials of the leaves extracts of *Kigelia africana, Baphia pubescens, Morinda lucida, Synclisa scabrida and Buchholzia coriaceae, Rauwolfia vomitoria, Nauclear latifolia, Anthocleista djalonensis* and *Moringa oleiferae.*

1.4 AIMS AND OBJECTIVES

1.4.1 Aim of study

The aim of this study is to investigate the pharmacognostic and anti-malarial properties of *B. pubescens* leaves.

1.4.2 Objectives of study

The specific objectives of this study include

- to assess the antimalarial activities of nine selected plants and to further investigate the most effective plant;
- to fractionate and to identify the secondary metabolite(s) responsible for the anti-malarial activity;
- and to study the toxicity and pharmacognostic standards of the plant.

CHAPTER TWO

LITERATURE REVIEW

2.1 MALARIA

2.1.1 History

Malaria is a disease caused by *Plasmodium* parasites that infect about 154 to 289 million people per year, resulting in approximately 660,000 - 2 million deaths worldwide (Vaughan *et al* 2008, Vogel, 2013). Symptoms of malaria include recurrent cycles (every one to three days) of fever, chills, muscle aches, headaches, nausea, vomiting, and jaundice (Bledsoe, 2005). Anopheles mosquitoes transmit the parasites to human when they bite. The parasites undergo a complicated life cycle in both mosquitoes and humans (Baird, 2013). The cycle repeats when the mosquitoes take a blood meal from a human who is contaminated with mature parasites (Richter *et al.,* 2010, Meremekwu *et al.,* 2012). Africa, Asia, and Central and South America are the areas with high numbers of malaria infections (Mens *et al.,* 2012).

Although the parasite responsible for *P. falciparum* malaria has been in existence for 50, 000-100, 000 years, the population size of the parasite did not increase until about 1000 years ago, concurrently with advances in agriculture (Harper and Armelagos, 2011; Korenromp *et al.,* 2005) and the development of human settlements. Close relatives of the human malaria parasites remain common in chimpanzees. Some evidence suggests that the *P. falciparum* malaria may have originated from gorilla (Prugnolle *et al.*, 2011).

References to the unique periodic fevers of malaria are found throughout recorded history, beginning in 2700 BC in China (Cox, 2002; Bray, 2004). Malaria may have contributed to the

decline of the Roman Empire, (Sallares, 2002; Bruce - Chwatt, 1980) and was so pervasive in Rome that it was known as the "Roman fever" (Sallares, 2002).

Several regions in ancient Rome were considered at-risk for the disease because of the favorable conditions present for malaria vectors. These included areas such as southern Italy, the island of Sardinia, the Pontine Marshes, the lower regions of coastal Etruria and the city of Rome along the Tiber River. The presence of stagnant water in these places was preferred by mosquitoes for biting grounds (Tan and Sung, 2008). Irrigated gardens, swamp-like grounds, run-off from agriculture, and drainage problems from road construction led to the increase of standing water (Hays, 2005).

The term malaria originated from Medieval Italian: malaria -"bad air"; the disease was formerly called ague or marsh fever due to its association with swamps and marshland (Reiter, 2000; Hay *et al.*, 2010). Malaria was once common in most of Europe and North America (Lindemann, 1999), where it is no longer endemic (Grazt, 2006; Machault *et al.*, 2011), though imported cases persist (Webb, 2009; Waters and Edstein, 2012).

Malaria was the most important health hazard encountered by U.S. troops in the South Pacific during World War II, where about 500,000 men were infected (Bray, 2004). According to Joseph Patrick Byrne, sixty thousand American soldiers died of malaria during the African and South Pacific campaigns (Byrne, 2008). Scientific studies on malaria made their first significant advance in 1880, when Charles Louis Alphonse Laveran – a French army doctor working in the military hospital of Constantine in Algeria observed parasites inside the red blood cells of infected people for the first time. He therefore proposed that malaria is caused by this organism, the first time a protist was identified as causing diseases (O'Brien *et al.*, 2011; Olaniyi, 2005;

Olatunji and Atolani, 2009). For this and later discoveries, he was awarded the 1907 Nobel Prize for Physiology/Medicine. A year later, Carlos finally, a Cuban doctor treating people with yellow fever in Havana, provided strong evidence that mosquitoes were transmitting the disease to and from humans (Tan *et al.,* 2008). This work followed earlier suggestions by Josiah C. Nott (Chernin, 1983,) and work done by Sir Patrick Manson, the "father of tropical medicine", on the transmission of filariasis (Chernin, 1977).

In April 1894, a Scottish physician Sir Ronald Ross visited Sir Patrick Manson at his house on Queen Anne Street, London. This visit was the start of four years of collaboration and fervent research that culminated in 1898 when Ross, who was working in the Presidency General Hospital in Calcutta, proved the complete life-cycle of the malaria parasite in mosquitoes. He thus proved that the mosquito was the vector for malaria in humans by showing that certain mosquito species transmit malaria to birds. He isolated malaria parasites from the salivary glands of mosquitoes that had fed on infected birds. For this work, Ross received the 1902 Nobel Prize in Medicine. After resigning from the Indian Medical service, Ross worked at the newly established Liverpool School of Tropical Medicine and directed malaria-control efforts in Egypt, Panama, Greece and Mauritius. The findings of Finlay and Ross were later confirmed by a medical Board headed by Walter Reed in 1900. Its recommendations were implemented by William C. Gorgas in the health measures undertaken during construction of the Panama Canal. This public - health work saved the lives of thousands of workers and helped develop the methods used in future public-health campaigns against the diseases (Simmons, 1979; Tenser *et al.,* 2010).

The first effective treatment for malaria came from the bark of Cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru (Srinivas *et al.,* 2007,

Perkins and Bell, 2008). The indigenous peoples of Peru made a tincture of cinchona to control the fever. Its effectiveness against malaria was found and the Jesuits introduced the treatment to Europe in 1640 and by 1677, it was included in the London pharmacopoeia as an anti-malarial treatment (Krotoski *et al*., 1982; Kumar *et al*., 2008). It was not until 1820 that the active ingredient, quinine was extracted from the bark, isolated and named by the French chemists Pierre Joseph Pelletier and Joseph Bienaime Cavantou (Kyle *et al.*, 1974).

Quinine became the predominant malarial medication until the 1920s, when other medications began to be developed. In the 1940s, chloroquine replaced quinine as the treatment of both uncomplicated and severe malaria until resistance supervened, first in Southeast Asia and South America in the 1950s and then globally in the 1980s (Achan *et al*., 2011, Bartonoloni. and Zammarchi, 2012). Artemisinins, discovered by Chinese scientist Tu Youyou and colleagues in the 1970s from the plant *Artemisia annua* became the recommended treatment for *P. falciparum* malaria, administered in combination with other antimalarials, to treat uncomplicated malaria as well as in severe disease (Hsu, 2006; Humphrey, 2001).

Plasmodium vivax was used between 1917 and the 1940s for malariotherapy, which is the deliberate injection of malaria parasites to induce fever to combat certain diseases such as tertiary syphilis (Feachem *et al* 2010, Fernando *et al*., 2010). In 1917, the inventor of this technique, Julius Wagner-Jauregg, received the Nobel Prize in Physiology/medicine for his discovery. The technique was dangerous killing about 15% of patients, so it is no longer in use (Vogel, 2013; Ferri, 2009).

The first pesticide used for indoor residual spraying was DDT. Although it was initially used exclusively to combat malaria, its use quickly spread to agriculture. In time, pest control, rather

than disease control, came to dominate DDT use, and this large-scale agricultural use led to the evolution of resistant mosquitoes in many regions. The DDT resistance shown by Anopheles mosquitoes can be compared to antibiotic resistance shown by bacteria. During the 1960s awareness of the negative consequences of its indiscriminate use increased, ultimately leading to bans on agricultural applications of DDT in many countries in the 1970s (Van der Berg, 2009; Wernsdorfer, 1980). Before DDT ban, malaria was successfully eliminated or controlled in tropical areas like Brazil and Egypt by removing or poisoning the breeding grounds of mosquitoes or the aquatic habitats of the larval stages, for example by applying the highly toxic arsenic compound - Paris Green to places with standing water (Killeen *et al*, 2002; Gbile *et al.*, 1993).

Malaria vaccines have been an elusive goal of research. The first promising studies demonstrating the potential for a malaria vaccine were performed in 1967 by immunizing mice with live, radiation-attenuated sporozoites, which provided significant protection to the mice upon subsequent injection with normal, viable sporozoites. Since the 1970s, there has been a considerable effort to develop similar vaccination strategies within humans (Vander Berg, 2009).

2.1.2 Malaria Statistics (Epidemiology)

The WHO estimated that in 2010 there were 219 million cases of malaria resulting in 660,000 deaths (Nadjm and Behrens, 2012). Others have estimated the number of cases at between 350 and 550 million for *falciparum* malaria (Olupot-Olupot *et al*, 2013) and deaths in 2010 at 1.24 million (Murray *et al.*, 2012); up from 1.0 million deaths in 1990 (Maegraith, 1973). The majority of cases (65%) occur in children under 15 years old (Murray *et al.*, 2012). About 125 million pregnant women are at risk of infection each year; in Sub-Saharan Africa, Maternal malaria is associated with up to 200,000 estimated infant deaths yearly (Hartman *et al.*, 2010).

There are about 10,000 malaria cases per year in Western Europe, and 1300-1500 in the United States (Taylor *et al.*, 2012). About 900 people died from the disease in Europe between 1993 and 2003 (Kajfasz, 2009). Both the global incidence of disease and resulting mortality has declined in recent years. According to the WHO, deaths attributable to malaria in 2010 were reduced by over a third, largely due to the widespread use of insecticide-treated nets and artemisinin - based combination therapies (Howitt *et al.*, 2012.)

Malaria is presently endemic in a broadband around the equator, in areas of the Americas, many parts of Asia, and much of Africa; in Sub-Sahara Africa, 85-90% of malaria fatalities occur (Layne, 2006). An estimate for 2009 reported that countries with the highest death rate per 100,000 of population were Ivory Coast (86.15), Angola (56.93) and Burkina Faso (50.66) (Provost, 2011). A 2010 estimate indicated that the deadliest countries per population were Burkina Faso, Mozambique and Mali (Murray *et al.*, 2012). The Malaria Atlas Project aims to map global endemic levels of malaria, providing a means with which to determine the global spatial limits of the disease and to assess disease burden (Guerra *et al.*, 2007; Hay *et al.*, 2010). This effort led to the publication of a map of *P. falciparum* endemicity in 2010 (Gething *et al.*, 2011). As of 2010, about 100 countries have endemic malaria (WHO, 2012; Feachem *et al.*, 2010). Every year, 125 million international travelers visit these countries, and more than 30,000 contract the disease (Kajfasz, 2009).

The geographic distribution of malaria within large regions is complex, and malaria – afflicted and malaria – free areas are often found close to each other (Greenwood and Mutabingwa, 2002). Malaria is prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity, along with stagnant waters in which mosquito larva readily mature, providing them with the environment they need for continuous breeding (Jamieson *et al.*,

2006). In drier areas, outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall (Abeku, 2007). Malaria is more common in rural areas than in cities. For example, several cities in the Greater Mekong Sub-region of Southeast Asia are essentially malaria –free, but the disease is prevalent in many rural regions, including along international borders and forest fringes (Cui *et al.*, 2012).

In contrast, malaria in Africa is present in both rural and urban areas, though the risk is lower in the larger cities (Machault *et al.*, 2011).

About 3.3 billion people – half of the world's population are at risk of malaria (Webb 2009). In 2010, there were about 219 million malaria cases (with an uncertainty range of 490, 000 to 289 million) and an estimated 660, 000 malaria deaths (with an uncertainty range of 490, 0000 to 836, 000). Increased prevention and control measures have led to a reduction in malaria mortality rates by more than 25% globally since 2000 and by 33% in the WHO Africa Region.

People living in the poorest countries are the most vulnerable to malaria. In 2010, 90% of all malaria deaths occurred in the WHO African Region, mostly among children under five years of age. In summery -

- Over half a million (655,000) people die from malaria each year, mostly children younger than five years old.
- There are an estimated 216 million cases of malaria each year.
- Although the vast majority of malaria cases occur in sub-Saharan Africa, the disease is a public- health problem in more than 109 countries in the world, 45 of which are in Africa.
- Approximately 3.3 billion people live in areas where malaria is a constant threat.
- 90% of all malaria deaths occur in sub-Saharan Africa.

- Malaria costs an estimated $12 billion in lost productivity in Africa.
- When insecticide–treated nets are used properly by three-quarters of the people in a community, malaria transmission is cut by 50%, child deaths are cut by 20%, and the mosquito population drops by as much as 90 %.
- It is estimated that less than 5% of children in sub-Sahara Africa currently sleep under any types of insecticide- treated net.

2.1.3 Life cycle of malaria parasite

Malaria parasites belong to the genus *Plasmodium* (phylum Apicomplexa). In humans, malaria is caused by *P. falciparum*, *P. malariae, P. ovale, P .vivax* and *P. knowlesi* (Mueller *et al*, 2007 and Collins, 2012). Among those infected, *P. falciparum* is the most common species identified (75%) followed by *P. vivax* (20%) (Nadjm and Behrens, 2012) Although *P. falciparum* traditionally accounts for the majority of death (Sarkar *et al*, 2009, Odugbami, 2008), recent evidence suggests that *P. Vivax* malaria is associated with potentially life-threatening conditions which often comes in combination with *P. falciparum* infection (Baired, 2013; Russell and Norman, 1998). *P. vivax* proportionally is more common outside of Africa (Arnott *et al.*, 2012; Arrow *et al.,* 2004) There have been documented evidence that human infections with several species of plasmodium emanate from higher animals such as apes, however with the exception of *P. knowlesi* - a zoonotic specie that causes malaria in macaques (Collins, 2012; Awe and Omojasola, 2003) – these are mostly of limited public health importance (Collins *et al.,* 2009).

The life cycle of the malaria parasite (*Plasmodium)* is complicated and involved two hosts, humans and Anopheles mosquitoes. The disease is transmitted to humans when an infected Anopheles mosquito bites a person and injects the malaria parasites (sporozoites) into the blood. Sporozoites travel through the bloodstream to the liver, mature, and eventually infect the human

red blood cells. While in red blood cells, the parasites again develop until a mosquito takes a blood meal from an infected human and ingest human red blood cells containing the parasites (Kattenberg *et al.,* 2011; Kaufman and Ruveda, 2005). The parasites reach the Anopheles mosquito's stomach and eventually invade the mosquito salivary glands. When an Anopheles mosquito bites a human, these sporozoites complete and repeat the complex *Plasmodium* life cycle. *P. ovale* and *P. vivax* can further complicate the cycle by producing dormant stages (hypnozoites) that may not develop for weeks to years. Malaria is not contagious from person to person. Malaria is transmitted to people by mosquitoes (Kokwaro, 2009; Idro *et al.,* 2010).

From the beginning, a female *Anopheles* mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate series of sexually multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew. Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilized mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form a ookinete – a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary glands ready to infect a new vertebrate host. The sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal (Cowman *et al.,* 2012; Keating, 2012).

Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and thus do not transmit the disease. The female of the *Anopheles* genus of mosquito prefer to feed at night. They

usually start searching for a meal at dusk, and will continue throughout the night until taking a meal (Arrow *et al.,* 2014). Malaria parasites can also be transmitted by blood transfusions; although this is rare (Owusu-Ofori *et al.,* 2010; Hall, 1976).

Transmission of malaria occurs through a vector, the mosquito that ingests gametocytes- the sexual form of the parasite – when feeding on an infected human. Gametocytes, which are both male and female, mate within the gut of the mosquito and undergo meiosis and then migrate through the mid gut wall of the mosquito and form an oocyst, within which thousand of sporozoites develop. These are then injected into human during the next blood meal(s), where they rapidly make their way to the liver and form hepatocytes and begin asexually (mitotically) replicating. After a period of 6-15 days, the liver schizonts rupture, releasing thousands of merozoites into the blood where they invade red blood cells. Over the next 48 hour, the parasite begins replicating mitotically, progressing through a set of stages (ring, trophozoite and schizont), and produce an average of 16 new daughter merozoites per schizont. The schizonts then burst in near synchrony with other parasites, producing the characteristics fever cycle that embodies the clinical manifestation of the diseases, with each replication, some of the merozites susceptible mosquitoes, bringing the transmission cycle to full circle.

The key to the life cycle of the malaria *Plasmodium* is the alternation of the two different host organisms, the invertebrates (anopheles mosquito) and a superior vertebrate (man). As long as these two hosts are available, the survival, of *Plasmodium* as a living species is ensured.

2.1.4 Invertebrate host phase

The female anopheles mosquito feeds on a malarial patient, ingesting human blood containing male (micro) and female (macro) gametocytes. In the mosquito's gut, the gametocytes emerge

from parasitized erythrocytes (red blood cells). The female gametocytes develops directly into a single macrogamete: the male gametocytes undergo vary rapid nuclear division and emits eight flagellate filament, each containing a nucleus, which then separate from the micro gametocyte body and develop into gametes: the whole process being influenced by temperature and other factors. This process lasts for 20 minutes. The microgamete, with the aid of the flagella, moves towards the macrogamete and penetrate it to form the zygote (fertilized ovum). The zygote develops into a mobile ookinete within twenty four hours (Jellife, 1966). The ookinete migrates towards the wall of the middle intestine, where sporozoites are eventually formed. These separates from the sporoblast buds, emerge free in the mosquito's coelom, and then concentrate in the insect's salivary glands, where they mature into infectious forms and then migrate to the salivary ducts, ready to be injected into man via bite (Jamison *et al.,* 2006). Overall, invertebrate stage takes between eight days and four weeks, the number of sporozoites produced ranging from one *Plasmodium* species to another (Wersndorfer, 1980).

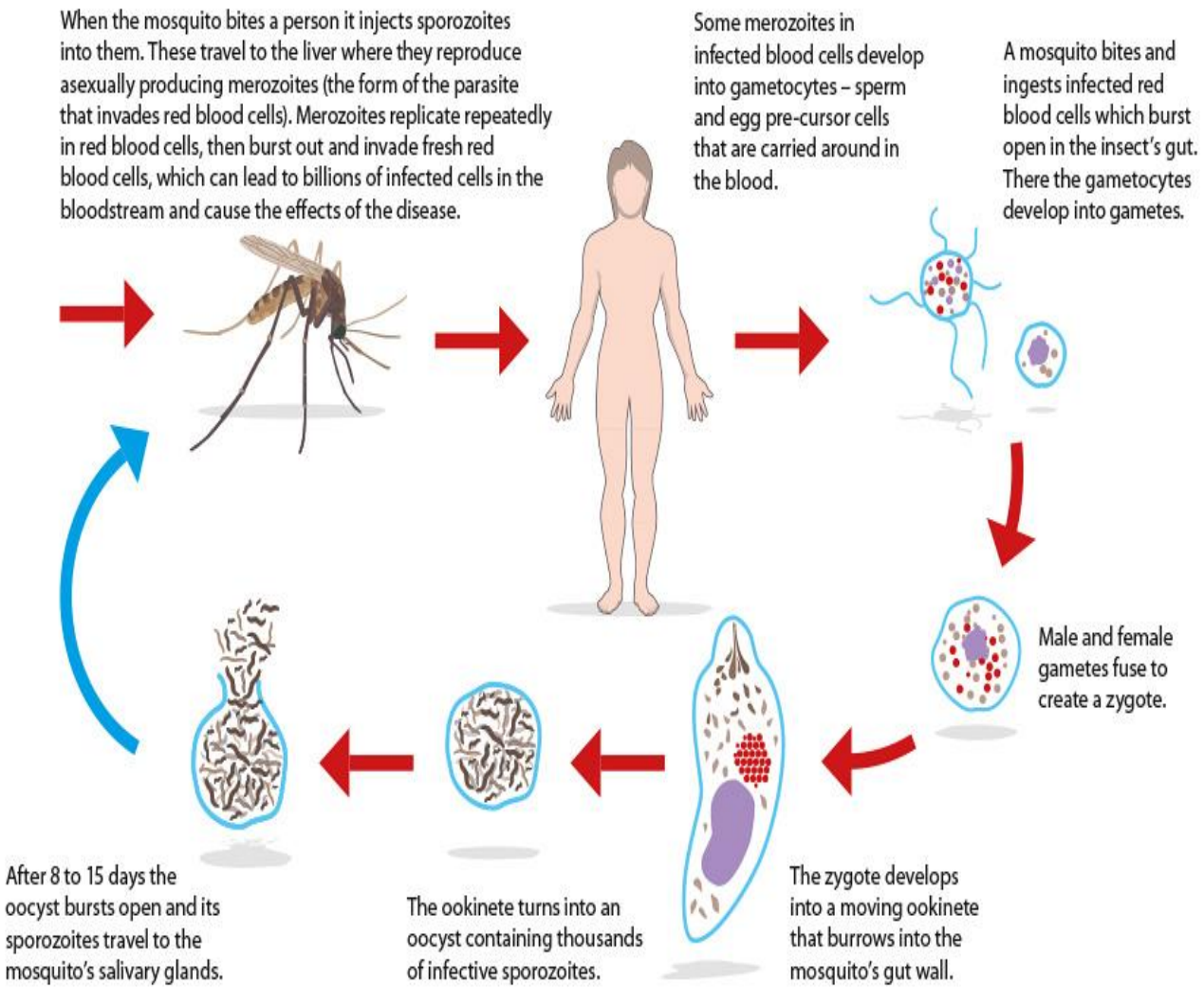


Figure 1: Life cycle of the parasite
https://mosquitoessuck.weebly.com/about-malaria.html (Date accessed 27.08.2018)

Table 1: Main features of the four species of *Plasmodium* pathogenic to man

Characteristics	***P. vivax***	***P. ovale***	***P. malariae***	***P. falciparum***
Duration of Sporogony (days)	8-9	12-14	14-15	10
Duration of the pre-erythrocytic cycle (days)	8	9	14	5
Presence of hypnozoites	Yes	Yes	No	No
Duration of erythrocytic cycle (hours)	48	50	72	48
Shape of gametocytes	Round	Round	Round	Crescent
Length of erythrocytic merozoite (µm)	1.2	1.8	-	0.7
No. of erythrocytic merozoites	8-24	4-8	8	8-32

2.1.5 Vertebrate host phase (asexual cycle/schizogonic cycle)

This stage also begins with a mosquito bite and the associated injection of sporozoites. These invade the blood stream and reach the liver within minutes (one hour at the most), where they initiate the pre-erythroytic phase.

The sporozoites begin to invade the Kupffer cells of the liver sinusoids, from where they penetrate adjoining liver cells in a matter of minutes (Meis *et al.,* 1983). The sporozoites then undergo transformation to form trophozoites, from which they form hypnozoites. The discovery of hypnozoites (Krotoski *et al.,* 1982) has introduced a new important link in the chain of plasmodial life cycle phases.

Generally, the period between the mosquito bite and the onset of the malarial illness is usually one to three weeks (7- 21 days). This initial time period is highly variable as reports suggest that range of incubation periods may be from four days to one year. The usual incubation period may be increased when a person has taken an inadequate course of malaria prevention medications. Certain types of malaria (*P. vivax and P. ovale*) parasites can also take much longer, as long as eight to 10 months, to cause symptoms (Kajifasz, 2009). These parasites remain dormant (inactive or hibernating) in the liver cells during this time. Unfortunately, some of these dormant parasites can remain even after a patient recovers from malaria, so the patient can get sick again. This situation is termed relapsing malaria (Krotoski *et al.,* 1982; Sitprija, 1970).

2.1.6 Relapsing (recurrent) malaria

Symptoms of malaria can recur after varying symptoms-free periods. Depending on the cause, recurrence can be classified either as recrudescence, relapse, or re- infection (Kyle and Shampe, 1974). Recrudescence is when symptoms return after a symptom–free period. It is caused by

parasites surviving in the blood as a result of inadequate or ineffective treatment (WHO, 2014). Relapse is when symptoms reappear after the parasites have been eliminated from blood but persist as dormant hypnozoites in liver cells. Relapse commonly occurs between 8-24 weeks and is commonly seen with *P.vivax* and *P. ovale* infections (Nadjm and Behrens, 2012; Lal *et al.*, 1998). *P. vivax* malaria cases in temperate areas often involve overwintering by hypnozoites, with relapse about a year after the mosquito bite (White, 2011). Re- infection means the parasite that caused the past infection was eliminated from the body but a new parasite was introduced. Re-infection cannot readily be distinguished from recrudescence, although recurrence of infection within two weeks of treatment for the initial infection is typically attributed to treatment failure (WHO, 2014). People may exhibit pre-immunity in case of recurrent infections (Maegraith, 1973).

2.1.7 Pathophysiology of malaria

As mentioned earlier, malaria infection develops via two phases: one that involves the liver (exo-erythrocytic phase), and one that involves red blood cells (erythrocytic phase) (Lalloo *et al.*, 2006). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8-30 days (Bledsoe, 2005; Riley and Stewart, 2013).

After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoite, which, following rupture of their host cells, escape into the blood and infect red blood cells to begin the erythrocytic stage of the life cycle (Bledsoe, 2005). The parasite escapes from

the liver undetected by wrapping itself in the cell membrane of the infected host liver cell (Vaughan *et al.*, 2008; Layne, 2006).

Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their host cells, to invade fresh red blood cells. Several such amplification cycles occur (US Department of Army, 1967). Thus, classical description of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells (Bledsoe, 2005)

Some *P. vivax* sporozoites do not immediately develop into exoerythrocytic-phase, - merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (7-10 months is typical) to several years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in *P. vivax* infections (White, 2011), although their existence in *P. ovale* is uncertain (Richer *et al.*, 2010).

The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen (WHO, 1973). To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the wall of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen (Tilley *et al.*, 2011). The blockage of the microvasculature causes symptoms such as in placental malaria (Mens *et al.*, 2012). Sequestered red blood cells can reach the blood – brain barrier and cause cerebral malaria (Renia *et al*., 2012).

Malaria may pose a serious threat to a pregnant woman and her fetus. Malaria infection in pregnant women may be more severe than in women who are not pregnant. Malaria may also

increase the risk of problems with the pregnancy, including prematurity, abortion, and stillbirth. Statistics indicate that in sub-Saharan Africa, between 75,000-200,000 infants die from malaria per year; worldwide estimates indicate about 2 million children die from malaria each year (Fairhurst and Wellems, 2010). Therefore, all pregnant women who are living in or travelling to a malaria risk area should consult a doctor and take prescription drug (for example, sulfadoxine–pyrimethamine) to avoid contracting malaria. Treatment of malaria in the pregnant female is similar to the usual treatment described above; however, drugs such as primaquine, tetracyclines and halofantrine (Halfan) are not recommended as they may harm the fetus. In addition to monitoring the patient for anemia, an Obstertrician/Gynacologist specialist often is consulted for further management (Owosu-Ofori *et al* 2010; Rijken *et al.,* 2012).

All children, including young infants, living in or traveling to malaria risk should take antimalarial drugs [for example, chloroquine and mefloquine (Lariam)]. Although the recommendations for most antimalarial drugs are the same as for adults, it is crucial to use the correct dosage for the child. The dosage of drug depends on the age and weight of the child. A specialist in pediatric infectious disease is recommended for consultation in prophylaxis (prevention) and treatment of children. Since an overdose of an antimalarial drug can be fatal. All anti-malarial (and all other) drugs should be stored in childproof containers well out of the child's reach.

2.1.8 Signs and symptoms of malaria

The signs and symptoms of malaria typically begin 8-25 days following infection (Fairhurst *et al*, 2010); however, symptoms may occur later in those who have taken anti-malaria medications as prevention (Nadjm and Behrens, 2012). Initial manifestations of the disease – common to all malaria species – are similar to flu-like symptoms (Bartoloni and Zammarchi, 2012), and can

resemble other conditions such as septicemia, gastroenteritis, and viral diseases (Nadjm and Behrens, 2012). The presentation may include headache, fever, shivering, joint pain, vomiting, hemolytic anemia, jaundice, hemoglobin in the urine, retinal damage, and convulsions (Beare *et al*, 2006).

Cycles of chills, fever, and sweating that repeat every one, two, or three days are the most typical symptoms. The classical textbook hot-cold stages (rarely observed) occur over about six to nine hours with a cold stage marked by shivering and feeling cold, which is then followed by a hot stage when fever, headaches, vomiting and occasionally seizures occur. Sweating also occurs and the patient becomes febrile, tired or exhausted. There can sometimes be vomiting, diarrhea, coughing, and yellowing (jaundice) of the skin and whites of the eyes due to destruction of red blood cells and liver cells, infrequently, a rash (skin lesions that are small itchy, reddish collections of papules) may occur.

People with severe *P. falciparum* malaria can develop bleeding problems, shock, liver or kidney failure, central nervous system problems, coma, and can die from the infection or its complications. Cerebral malaria (coma, or altered mental status or seizures) can occur with severe *P. falciparum* infection. It is lethal if not treated quickly; even with treatment, about 15% - 20% die (Reiter, 2000).

The classic symptoms of malaria is paroxysmal (a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating), occurring every two days in Tertian fever in *P. vivax* and *P. ovale* infections and every three days (quartan fever) for *P. malariae*. *P. falciparum* infection can cause recurrent fever every 36-48 hours or a less pronounced and almost continuous fever (Ferri, 2009).

Severe malaria is usually caused by *P. falciparum* (often referred to as *falciparum* malaria). Symptoms of *falciparum* malaria arise 9- 30 days after infection (Byrne, 2008). Individuals with cerebral malaria frequently exhibit neurological symptoms, including abnormal posturing, nystagmus, conjugate gaze palsy (failure of the eyes to turn together in the same direction), opisthotonos, seizures, or coma (Bartoloni and Zammarchi, 2012).

2.1.9 Complications of malaria

There are severe serious complications of malaria. Among these is the development of respiratory distress, which occurs in up to 25% of adults and 40% of children with severe *P. falciparum* malaria. Possible causes include respiratory compensation of metabolic acidosis, non - cardiogenic pulmonary edema, concomitant pneumonia, and severe anemia. Although rare in young children with severe malaria, acute respiratory distress syndrome occurs in 5-25% of adults and up to 29% of pregnant women (Taylor *et al.*, 2012). Co-infection of HIV with malaria increases mortality (Korenromp *et al.*, 2005). Renal failure is a feature of black water fever, where hemoglobin from lysed red blood cells leaks into the urine (Bartoloni and Zammarchi, 2012).

Infection with *P. falciparum* may result in cerebral malaria, a form of severe malaria that involves encephalopathy. It is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever (Beare *et al.*, 2011). Splenomegaly, severe headache, hepatomegaly (enlarge liver), hypoglycemia, and hemoglobinuria with renal failure may occur (Bartoloni and Zammarchi, 2012).

Malaria is classified into either “severe” or “uncomplicated” by the World Health Organization (Nadjm *et al.*, 2012). It is deemed severe when any of the following criteria are present, otherwise it is considered uncomplicated.

- Decreased consciousness
- Significant weakness such that the person is unable to walk
- Inability to feed
- Two or more convulsions
- Low blood pressure (less than 70 mmHg in adults and 50 mmHg in children)
- Breathing problems
- Circulatory shock
- Kidney failure or hemoglobin in the urine
- Bleeding problems, or hemoglobin less than 50 g/L (5g/dL)
- Pulmonary oedema
- Blood glucose less than 2.2 mmol/L (40mg /dL)
- Acidosis or lactate level of greater than5 mmol/L
- A parasite level in the blood of greater than 100, 000 /µL in low-intensity transmission areas, or 250,000 per µL in high –intensity transmission areas.

Cerebral malaria is defined as a severe *P. falciparum* – malaria presenting with neurological symptoms, including coma (with a Glasgow coma scale less than 11, or a Blantyre coma scale greater than 3), or with a coma that lasts longer than 30 minutes after a seizures.

Malaria in pregnant women is an important cause of stillbirths, infant mortality and low birth weight (Hartman *et al.*, 2010), particularly in *P. falciparum* infection, but also with *P. vivax* (Rijken *et al.*, 2012).

2.1.10 Prognosis

The majority of people who become infected with *P. malariae, vivax,* or *ovale* do well and the fevers abate after about 96 hours. However, in endemic areas, re-infection is common. Malaria caused by *P. falciparum* or *P. knowlesi,* even when treated, have outcomes ranging from fair to poor, depending on how the parasites react to treatment. Untreated people often die from these infections. In general, patients who are infants, children under the age of 5 (especially in sub-Saharan countries), and those with depressed immune systems (for example, AIDS or cancer patients) have a more guarded prognosis.

When properly treated, people with malaria can usually expect a complete recovery. However, severe malaria can progress extremely rapidly and cause death within hours or days (Trampuz *et al.,* 2003). In the most severe cases of the disease, fatality rates can reach 20% even with intensive care and treatment (Nadjm and Behrens, 2012). Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria (Fernando *et al,* 2010). Chronic infection without severe disease can occur in an immune-deficiency syndrome associated with a decreased responsiveness to *Salmonella* bacteria and the *Epstein- Barr*-virus (Riley and Stewart, 2013).

During childhood, malaria causes anemia during a period of rapid brain development, and also direct brain damage resulting from cerebral malaria (Fernando *et al.*, 2010). Some survivors of cerebral malaria have an increased risk of neurological and cognitive deficits, behavioral

disorders, and epilepsy (Idro *et al.,* 2010). Malaria prophylaxis was shown to improve cognitive function and school performance in clinical trials when compared to placebo groups (Fernando *et al.*, 2010).

2.1.11 Diagnosis of malaria

Clinical symptoms associated with travel to countries that have identified malarial risk suggest malaria diagnosis. Malaria tests are not routinely ordered by most physicians so recognition of travel history is essential. Unfortunately, many disease can mimic symptoms of malaria (for example, yellow fever, dengue fever, typhoid fever, cholera, filariasis, and even measles and tuberculosis). Consequently, physicians need to order the correct special tests to diagnose malaria, especially in industrialized countries where malaria is seldom seen. Without the travel history, it is likely that other tests will be ordered initially. In addition, the long incubation periods may tend to allow people to forget the initial exposure to infected mosquitoes (Powell, 1972).

Due to the non- specific nature of the presentation of symptoms, diagnosis of malaria in non-endemic areas requires a high degree of suspicion, which might be elicited by any of the following recent travel history, enlarge spleen, fever, low number of platelets in the blood, and higher –than –normal levels of bilirubin in the blood combined with a normal level of white blood cells (Nadjm and Behrens, 2012).

Malaria is usually confirmed by the microscopic examination of blood films or by antigen –based rapid diagnosis tests (RDT) (Abba *et al.,* 2011 and Kattenberg *et al.*, 2011). Microscopy is the commonly used method to detect the malaria parasites – about 165 million blood film were examined for malaria in 2010 (Wilson, 2012). Despite its widespread usage, diagnosis by

microscopy suffers from two main drawbacks: many settings (especially rural) are not equipped to perform the test, and the accuracy of the results depends on both the skill of the person examining the blood film and the levels of the parasite in the blood. The sensitivity of blood films ranges from 75-90% in optimum conditions, to as low as 50 %. Commercially available RDTs are often more accurate than blood films at predicting the presence of malaria parasites, but they are widely variable in diagnostic sensitivity and specificity depending on manufacturer, and are unable to tell how many parasites are present (Wilson, 2012, Worrall *et al.,* 2005).

In region where laboratory tests are readily available malaria should be suspected, and tested for, in any patient who has been in an area where malaria is endemic. In areas that cannot afford laboratory diagnostic tests, it has become routine to use only a history of subjective fever as the indication to treat for malaria – a presumptive approach exemplified by the common teaching "fever equals malaria unless proven otherwise". A drawback of this practice is over – diagnosis of malaria and mismanagement of no- malarial fever, which wastes limited resources, erodes confidence in the health care system, and contributes to drug resistance (Perkins *et al.*, 2008). Although polymerase chain reaction – based tests have been developed, these are not widely implemented in malaria - endemic region as of 2012, due to their complexity (Nadjm and Behrens, 2012; Peters *et al.*, 1975).

The classic and most used diagnostic test for malaria is the blood smear on a microscopy slide that is stained (Giemsa stain) to show the parasites inside red blood cells (Raghavendra *et al.*, 2011).

Although this test is easily done, correct results are dependent on the technical skill of the laboratory technician who prepares and examine the slides with a microscope. Other tests based

on immunologic principles exist; including RDTs (rapid diagnostic tests) approved for use in the U.S. in 2007 and polymerase chain reaction (PCR) tests. These are not yet widely available and are more expensive than the traditional Giemsa blood smear (Peters, 1980).

2.1.12 Treatment of malaria

Three main factors determine treatments: the infecting species of *Plasmodium* parasite, the clinical situation of the patient (for example, adult, child, or pregnancy female with either mild or severe malaria), and the drug susceptibility of the infecting parasites (Provost, 2011). Drug susceptibility is determined by the geographic area where the infection was acquired. Different areas of the world have malaria types that are resistant to certain medications. The correct drugs for each type of malaria must be prescribed by a doctor who is familiar with malaria treatment protocols. Since people infected with *P. falciparum* malaria can die (often because of delayed treatment), immediate treatment for *P. falciparum* malaria is necessary (Renia *et al.*, 2012; Sheehy and Reba, 1976).

Mild malaria can be treated with oral medication; severe malaria (one or more symptoms of either impaired consciousness/coma, severe anemia, renal failure, pulmonary edema, acute respiratory distress syndrome, shock, disseminated intravascular coagulation, spontaneous bleeding, acidosis, hemoglobinuria (hemoglobin in the urine), jaundice, repeated generalized convulsion, and /or parasitemia (parasites in the blood) of >5%) requires intravenous (IV) drug treatment and fluid in the hospital (Blount, 1967).

Malaria is treated with antimalarial medications; the ones used depend on the type and severity of the disease. While medications against fever are commonly used, their effects or outcomes are not clear (Meremikwu *et al.*, 2012).

Uncomplicated malaria may be treated with oral medications. The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin – combination therapy, or (ACT), which decreases resistance to any single drug component (Kokwaro, 2009). These additional antimalarials include: amodiaquine, lumefantrine, mefloquine or sulfadoxine/primethamine (WHO, 2014). Another recommended combination is dihydroartemisnin and piperaquine (Keating, 2012). ACT is about 90% effective when used to treat uncomplicated malaria (Howitt *et al.*, 2012). To treat malaria during pregnancy, the WHO recommends the use of quinine plus clindamycin early in the pregnancy (1st trimester), and ACT in later stages (2nd and 3rd trimesters) (Manyando *et al.*, 2011). In the 2000s (decade), malaria with partial resistance to artemisinins emerged in Southeast Asia (O'Brien *et al.***,** 2011; Fairhurst *et al.*, 2012).

Infection with *P. vivax*, *P. ovale* or *P. malariae* is usually treated without the need for hospitalization. Treatment of *P. vivax* requires both treatment of blood stages (with chloroquine or ACT) as well as clearance of liver forms with primaquine (Waters and Edstein, 2012; Ricci, 2012).

Recommended treatment for severe malaria is the intravenous use of antimalarial drugs. For severe malaria, artesunate is superior to quinine in both children and adults (Sachs and Malaney 2002). Treatment of severe malaria involves supportive measures that are best done in critical care unit. This includes the management of high fevers and the seizures that may result from it. It also includes monitoring for poor breathing effort, low blood sugar, and low blood potassium (Sarkar *et al.*, 2009).

Drug treatment of malaria is not always easy. Chloroquine phosphate (Aralen) is the drug of choice for all malarial parasites except for chloroquinine –resistant *Plasmodium* strains. Although almost all strains of *P. malariae* are susceptible to chloroquine, *P. falciparum, P. vivax* and even some *P. ovale* strain have been reported as resistant to chloroquine. Unfortunately, resistant is usually noted by drug – treatment failure in the individual patient. There are, however, multiple drug-treatment protocols for treatment of drug-resistant *Plasmodium* strains (for example, quinine sulfate plus or tetracycline, or clindamycin, or atovaquone-proguanil).

There are specialized laboratries that can test the patient's parasites for resistance, but this is not done frequently (Burns 1966). Consequently, treatment is usually based on the majority of *plasmodium* species diagnosed and its general drug-resistance pattern for the country or world region where the patient became infected. For example, *P. falciparum* acquired in the Middle East countries is usually susceptible to chloroquine, but if it's acquired in sub-Sahara African countries, it's usually resistant to chloroquine.

The treatment policy, recently established in 2006 by the World Health Organization, is to treat all cases of uncomplicated *P. falciparum* malaria with artemisinin-derived combination therapy (ACTS). ACTs are drug combinations (for example, artesunate-amodiaquine, artesunate–mefloquine, artesunate–pyronaridine, dihydroartemisinin-piperaquinin, and chlorproguanil – dapsone-artesunate) used to treat drug – resistant *P. falciparum*. Unfortunately, as of 2009, a number of *P. falciparum* –infected individuals have parasites resistant to ACT drugs.

New drug treatments of malaria are currently under study because *Plasmodium* species continue to produce resistant strains that frequently spread to other areas. One promising drug class under

investigation is the spiroindolones, which have been effective in stopping *P. falciparum* experimental infections (Daso, 2015).

Different antimalarials exert their effects at different stages of the parasite's life cycle. Chemoprophylaxis or therapy of acute attacks by chemosuppression of the clinical manifestations of the disease – or the elimination of all parasites at all stages (a radical cure) - may be achieved by drugs attacking at -

(1) the pre-erythrocytic stages (sometime called "primary exoerythrocytic" or "tissue" stages), which take place in the liver;

(2) the erythrocytic stages, in which the parasites multiply rapidly in erythrocytes; or

(3) the exoerythrocytic or secondary tissue stages of the relapsing malarias *(P vivax, P malariae, and P. ovale) w*hich also take place in the liver.

In addition, some drugs may have a selectively toxic effect on the gametocytes that form in the erythrocytes and are responsible for transferring the infection to the mosquito. Other drugs, when ingested in the blood by the mosquito, may prevent the transmission of malaria to another victim by preventing multiplication (sporogony) in the mosquito gut and salivary glands.

Antimalarial drugs are sometimes classified as follows:

(1) **Primary tissue schizonticides:** drugs that destroy the primary (pre-erythrocytic) tissue schizonts in the liver soon after infection (e.g, primaquine).
(2) **Blood schizonticides:** ("Chemosuppressive" or "clinically curative" drugs.) drugs that suppress the symptoms of malaria by destroying the schizonts and merozoites in the erythrocytes (e.g. quinine, quinacrine, chloroquine, and amodiaquine).

(3) **Gametocides:** drugs that prevent infection of mosquitoes and therefore the spread of infection by mosquitoes by destroying gametocytes in the blood (eg, primaquine).

(4) **Sporonticides:** Drugs that could help to eradicate the disease by preventing sporogony and multiplication of the parasites in the mosquito when ingested with the blood of the human host (eg, chloroguanide and pyrimethamine).

(5) **Secondary tissue schizonticides (**"Radically curative" drugs): Drugs use to cure the chronic relapsing fevers due to infection by *P vivax, P malariae, and P ovale* by destroying the secondary (exoerythrocytic) tissue schizonts developing in the liver (eg, primaquine).

2.1.13 Prevention and control of malaria

People travelling to malaria endemic areas need to have in their possessions antimalarial medications, which are to be taken according to prescription. Current CDC recommendations, for malaria prevention, suggest individuals begin taking anti-malarial drug (tablets) about one to two weeks before travelling to a malaria infested areas and for four weeks after leaving the areas (additional prophylactic or preventative therapy). Doctors, travel clinics, or the health department can advise individuals as to what medicines to take to keep them from getting malaria. Currently, there is no vaccine available for malaria, but researchers are trying to develop one (Sabot *et al*., 2010).

Methods used to prevent malaria include medications, mosquito elimination and the prevention of bites. The presence of malaria in an area requires a combination of high human population density, high anopheles mosquito population density and high rates of transmission from humans to mosquitoes and from mosquito to humans. If any of these is lowered sufficiently, the parasite will eventually disappear from that area, as happened in North America, Europe and parts of the Middle East (Tusting *et al*., 2013). However, unless the parasite is eliminated from the whole

world, it could become re-established if conditions revert to a combination that favours the parasite's reproduction. Furthermore, the cost per person of eliminating anopheles mosquitoes rises with decreasing population density, making it economically unfeasible in some areas (WHO, 1958; Tran *et al.*, 2012).

Many researchers argue that prevention of malaria may be more cost-effective than treatment of the disease in the long run, but the capital costs required are out of reach of many of the world's poorest people. There is a wide difference in the cost of control (i.e. maintenance of low endemicity) and elimination programmes between countries. For example, in China – whose government in 2010 announced a strategy to pursue malaria elimination in the Chinese provinces- the required investment is a small proportion of public expenditure on health. In contrast, a similar program in Tanzania would cost an estimated one –fifth of the public health budget (Sabot *et al.*, 2010).

Avoid travel to or through countries where malaria occurs if possible. If people must go to areas where malaria occurs, they should take all of the prescribed preventive medicine. In addition, the 2010 CDC- Committee on Disease Control-international travel recommendations suggest that the following precautions be taken in malaria and other disease–infected areas of the world. The following CDC recommendations are not unique for malaria but are posted by the CDC in their malarial prevention publication.

- Avoid outbreaks: To the extent possible, travelers should avoid traveling to areas of known malaria outbreaks. The CDC Travelers' Health web page provides alerts and information on regional disease transmission patterns and outbreak alerts (htt://www.cdc.gov/travel).

- Be aware of peak exposure times and places: exposure to arthropod bites may be reduced if travelers modify their patterns of activity or behavior. Although mosquitoes may bite at any time of the day, peak biting activity for vectors of some disease (for example, dengue, chikungunya, etc) is during daylight hours. Vectors of other disease (for example, malaria) are most active in twilight periods (for example, dawn and dusk) or in the evening after dark. Avoiding the outdoors or focusing preventive actions during peak hours may reduce risk.
- Wear appropriate clothing: Travelers can minimize areas of exposed skin by wearing long-sleeved shirts, long pants, boots and hats. Tucking in shirts and wearing socks and closed shoes instead of sandals may reduce risk. Repellents or insecticides such as pyrethrin can be applied to clothing and gear for added protection; this measure is discussed in detail below.
- Check for ticks: Travelers should be advised to inspect themselves and their clothing for ticks during outdoor activity and at the end of the day. Prompt removal of attached ticks can prevent some infections.
- Beds nets: when accommodations are not adequately screened or air conditioned, bed nets are essential to provide protection and to reduce discomfort caused by biting insects. If bed nets do not reach the floor they should be tucked under mattresses. Bed nets are most effective when they are treated with an insecticide or repellent such as pyrethrin. Pretreated, long-lasting bed nets can be purchased prior to traveling, or nets can be treated after purchase. The pyrethrin will be effective for several months if the bed net is not washed (long-lasting pretreated nets may be effective for much longer).
- Insecticides: Aerosol insecticides, vaporizing mats, and mosquito oils can help to clear rooms or areas of mosquitoes; however, some product available internationally may contain

pesticides that are not registered in the United State. Insecticides should always be used with caution, avoiding direct inhalation of spray or smoke.

- Optimum protection can be provided by applying repellents. The CDC recommended insect repellant should contain up to 50 % DEET (N, N-diethyl-meta-toluamide), which is the most effective mosquito repellent for adults and children over 2 months of age.

Vector control refers to methods used to decrease malaria by reducing the levels of transmission. For individual protection, the most effective insect repellents are based on DEET or picaridin (Kajfasz, 2009). Insecticide –treated mosquito nets (ITNs) and indoor residual spraying (IRS) have been shown to be highly effective in preventing malaria among children in areas where malaria is common (Lengeler, 2014, Tanser *et al.*, 2010).

IRS is the practice of spraying insecticides on the interior walls of homes in malaria-affected areas. After feeding, many mosquito species rest on a nearby surface while digesting the blood meal, so if the walls of houses have been coated with insecticides, the resting mosquitoes can be killed before they can bite another person and transfer the malaria parasite (Enayati *et al.*, 2007; McCann *et al.*, 1975). As of 2006, the World Health Organization recommends 12 insecticides in IRS operations, including dichlorodiphenyltrichloroethane (DDT) and the pyrethroids, cyfluthrin and deltamethrin. This public health use of small amounts of DDT is permitted under the Stockholm Convection on Persistent Organic Pollutants (POPs), which prohibits its agricultural use (Van der Berg, 2009; Enayati and Hemingway, 2010).

One problem with all forms of IRS is insecticide resistance. Mosquitoes affected by IRS tend to rest and live indoors, and due to the irritation caused by spraying, their descendants tends to rest and live outdoors, meaning that they are less affected by the IRS (Pates and Curts, 2005).

Mosquito nets create a protective barrier against malaria –carrying mosquitoes that bite at night. Mosquito nets help keep mosquitoes away from people and reduce infection rates and transmission of malaria. Nets are not a perfect barrier and are often treated with an insecticide designed to kill the mosquito before it has time to find a way past the net. Insecticide-treated nets are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net (Raghavendra *et al.*, 2011). Between 2000 and 2008, the use of ITNs saved the lives of an estimated 250,000 infants in Sub-Saharan Africa (Howitt *et al.*, 2012). Although ITNs prevent malaria, only about 13% of households in Sub-Sahara countries own them (Murray *et al.*, 2012). A recommended practice for usage is to hang a large "bed net" above the center of a bed to drape over it completely with the edges tucked in. Pyrethroid-treated nets and long-lasting insecticide-treated nets offer the best personal protection, and are most effective when used from dusk to dawn (Meis *et al.*, 1983, Melhorn, 2008).

There are a number of other methods intended to reduce mosquito bites and slow the spread of malaria. Efforts to decrease mosquito larva via decreasing the availability of open water in which they develop or by adding substances to decrease their development are effective in some locations (Tusting *et al.*, 2013). Electronic mosquito repellent devices which make very high frequency sounds that are supposed to keep female mosquitoes away, do not have supporting evidence (Enayati *et al.*, 2007).

Community participation and health education strategies promoting awareness of malaria and the importance of control measures have been successfully used to reduce the incidence of malaria in some areas of the developing world (Lallo *et al.*, 2006). Recognizing the disease in the early stages can stop the disease from becoming fatal. Education can also inform people to cover over areas of stagnant, still water, such as water tanks that are ideal breeding grounds for the parasite

and mosquito, thus cutting down the risk of the transmission between people. This is generally used in urban areas where there are large centers of population in a confined space and transmission would be most likely in these areas (Mehlhorn, 2008). Intermittent preventive therapy is another intervention that has been used successfully to control malaria in pregnant women and infants (Basir *et al.*, 2012) and in preschool children where transmission is seasonal (Meremikwu *et al.*, 2012).

Antimalaria Month is observed every year in the month of June by the National vector borne Disease Control programme (NVBDCP) of India, with an objective to increase multi-sectoral collaboration and community involvement in malaria control (Lal *et al.*, 1998).

2.1.14 Malaria vaccine

Currently, there is no commercially available vaccine for malaria. However, in the past few years, researchers have made good progress with experimental vaccine. A recent large clinical trial (in children in eight African countries) sponsored by the Gates foundation indicated the experimental vaccine protected about 50% of those immunized against malaria (WHO, 2013). In August 2013, a vaccine produced by Sanaria, Inc., and tested by the National Institute of Allergy and infectious disease reported a 100% protection rate in a small study group (CNN, 2013). This is the first time that a group of people have been effectively protected against malaria. However, this vaccine uses multiple IV injection of irradiated, frozen parasite, and various researchers suggest it may take eight to 10 years before the vaccine could be approved for commercial use (CNN, 2013). Other researchers are taking a novel approach to stopping malaria by interrupting the life cycle of the parasite using a vaccine in humans. Briefly, humans are vaccinated against a mosquito gut protein (named AnPNI) so the human produces antibodies against it. The mosquito bites the immunized human, ingests AnPNI antibodies while it sucks blood that in turn attack the

mosquito gut protein. The gut protein is required for the malaria parasite to develop, so mosquitos that get the antibodies no longer can transmit the disease to humans (Jumeper, 1978). In the future, we may see combination of such experimental vaccine methods to substantially reduce the devasting effect malaria parasite have on people, especially children. World Health Organization estimates for 2010, an average of 219 million people (154 to 289 million) was infected with 660,000 deaths world wide (the majority of deaths occurred in children).

Immunity (or, more accurately, tolerance) to *P. falciparum* malaria does occur naturally, but only in response to years of repeated infection (Tran *et al.*, 2012). An individual can be protected from a *P. falciparum* infection if they receive about a thousand bites from mosquitoes that carry a version of the parasite rendered non-infective by a dose of x-ray irradiation. An effective vaccine is not yet available for malaria, although several are under development (Geels *et al.*, 2011). The highly polymorphic nature of many *P. falciparum* proteins results in significant challenges to vaccine design. Vaccine candidates that target antigens, gametes, zygote or ookinetes in the mosquito mid gut, aim to block the transmission of malaria. These transmission blocking vaccine antibodies in the human blood; when a mosquito takes a blood meal from a protected individual, these antibodies prevent the parasite from completing its development in the mosquito (Crompton *et al.*, 2010).

Other vaccine candidates, targeting the blood-stage of the parasite's life cycle, have been inadequate on their own (Graves and Gelband, 2006). For example, SPf66 (pre-erythrocytic attenuated vaccine) was tested extensively in endemic areas in the 1990s, but clinical trials showed it to be insufficiently effective (Graves and Gelband, 2006). Several potential vaccine targeting the pre-erythrocytic stage of the parasite's life cycle are being developed, with RTS,S as the leading candidate (Hoffman *et al.*, 2010); it is expected to be licensed in 2015 (Riley and

Stewart, 2013). A US biotechnology company Sanaria, is developing a pre-erythrocytic attenuated vaccine called PfSPZ that uses whole sporozoites to induce an immune response (Hoffman *et al.*, 2010). In 2006, the malaria vaccine Advisory Committee to the WHO outlined a "Malaria Vaccine Technology Roadmap" that has as one of its landmark objectives to "develop and license a first – generation malaria vaccine that has a protective efficacy of more than 50% against severe disease and death and lasts longer than one year" by 2015.

2.1.15 Antimalarial Drug Resistance

A WHO scientific committee defined plasmodial drug resistance thus, "the ability of a parasite strain to survive and / or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject" (WHO, 1973).

Interestingly, the parasite showing resistance more often is the *P.falciparum.* This is the most widespread, and virulent of all plasmodial strains. This strain is capable of resisting the actions of almost all the antimalarial drugs available in the market (Harper and Armelagos, 2011).

Malarial parasites have many stages of development. Not all drugs are effective in these various stages. See table below

Table 2: **Stages of Antimalarial drug action**

Chemical class	Drug	Sporozoites	Tissue stage		Blood stage		Development of gametocytes in mosquito
			Primary	Secondary	Asexual	Sexual	
Cinchona alkaloids	Quinine	No action	No action	No action	Fast action	Active on *P. vivax ovale, mararіae*	No action
9-amino acridine	Mepacrine	No action	No action	No action	Fast action	Active on *P. vivax, ovale, malariae*	No action
4-amino quinolines	Chloroquine amodiaquine	No action	No action	No action	Fast action	Active on *P. vivax, ovale, malariae*	No action
8-amino quinolines	Primaquine	No action	Active but for prophylatic	Highly active	Active only in toxic disease	Active on all species	Active
Biguanides	Proguanil	No action	Active particularly on *P. falciparum*	Probably no action	Slow action	No direct action	Active
Diaminopyrimidines	Pyrimethamine	No action	Active particularly on *P. falciparum*	Probably no action	Slow action	No direct action	Active
Sulfomes sulfonamides	Dapsone	No action	Probably no action	Probably no action	Slow action	No direct action	Possible action
Sulfan tillolates	Dapsone + proguanil	No action	Active	Some action	Fast action	No direct	Active
Antibiotics	Minocycloine	No action	Active on *P. falciparum* but not adviced	No action	Slow action	No action	No action

2.1.16 Economic impact of malaria

Malaria is not just a disease commonly associated with poverty: some evidence suggests that it is also a cause of poverty and a major hindrance to economic development (Ryley and Peters, 1970; Worral *et al.*, 2005). Although tropical regions are most affected, malaria's furtherest influence reaches into some temperate zone that have extreme seasonal changes. The disease has been associated with major negative economic effects on regions where it is widespread. During the late 19th and early 20th centuries, it was a major factor in the slow economic development of the American southern states (Humphreys, 2001; Ohadome, 2016).

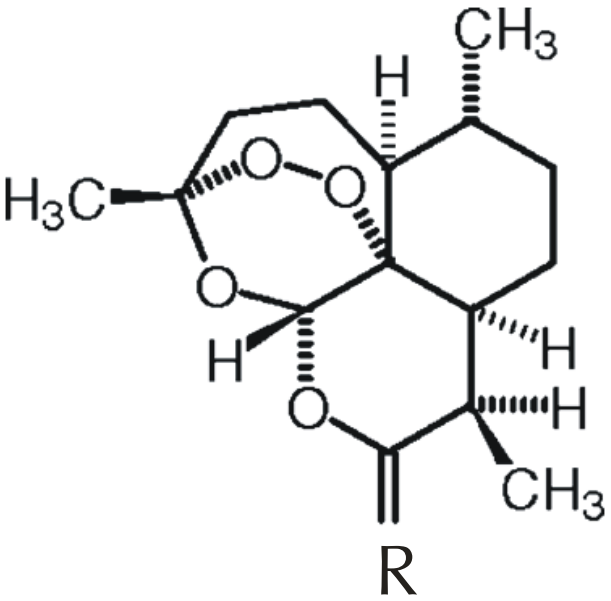
A comparison of average per capita gross domestic product (GDP) in 1995, adjusted for parity of purchasing power, between countries with malaria and countries without malaria gives a fivefold difference ($1,526 USD versus $8, 268 USD). In the period 1965 to 1990, countries where malaria was common had an average per capita GDP that increased only 0.4% year, compared to 2.4% per year in other countries (Sachs and Malaney, 2002).

Poverty can increase the risk of malaria, since those in poverty regions do not have the financial capacities to prevent or treat the disease. In its entirety, the economic impact of malaria has been estimated to cost Africa $12 billion USD every year. The economic impact includes costs of health care, working days lost due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism (Greenwood *et al.*, 2005). The disease has a heavy burden in some countries, where it may be responsible for 30-50% of hospital admissions, up to 50% of outpatient visits, and up to 40% of public health spending (WHO, 2003).

Cerebral malaria is one of the leading causes of neurological disabilities in Africa children (Idro *et al.,* 2010). Studies comparing cognitive functions before and after treatment for severe malarial illness continued to show significantly impaired school performance and cognitive abilities even after recovery (Fernando *et al.,* 2010). Consequently, severe and cerebral malaria have far-reaching socioeconomic consequences that extend beyond the immediate effects of the disease (Ricci, 2012).

2.2 PLANTS AS A SOURCE OF ANTIMALARIAL DRUG

Medicinal plants are plants whose extracts could be used directly or indirectly for the treatment of different diseases. The traditional use of medicinal plants in most developing countries as a basis for the maintenance of good health has been observed. According to the World Health Organization (WHO, 1977), medicinal plants are described as plants containing one or more structural organs with active principles that could be used for therapeutic purposes, as lead compounds or precursors for drug synthesis. Scientists are trying to explore the precious assets of medicinal plants to help the suffering humanity. Many pharmaceutical preparations are based on plants (Abbiw, 1990). An analogy could be drawn from the Chinese herb, *Artemisia annua* L., which has been utilized for over 100 years for malaria chemotherapy in China. From the plant was isolated a phytochemical constituent called artemisinin, a sesquiterpene lactone with an intramolecular peroxide linkage (Olaniyi, 2005). Structural modification of the sesquiterpene lactone moiety yielded artemisinin analogues with improved pharmacokinetic profile, hence improved bioavailability.



R= O, Artemisinin
R= OH, Dihydroartemisinin
R= OCH3 Artemether
R= OCHOH Arteether
R= OCO(CH2)2COOH Artesunate

Figure 2: Sesquiterpene lactone ring structure

Table 3: Structural Formulas of Antimalarial Drugs

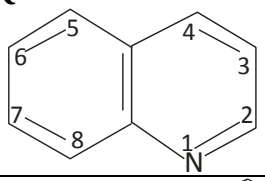
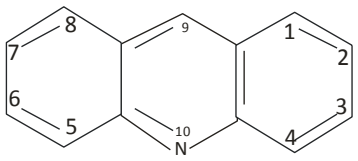
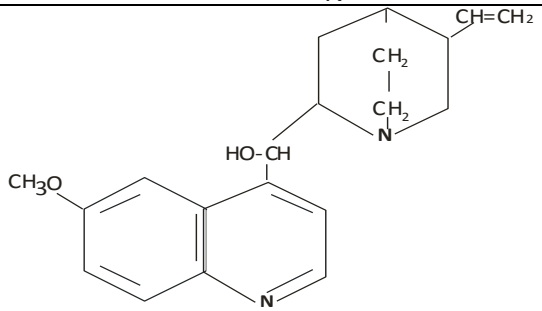
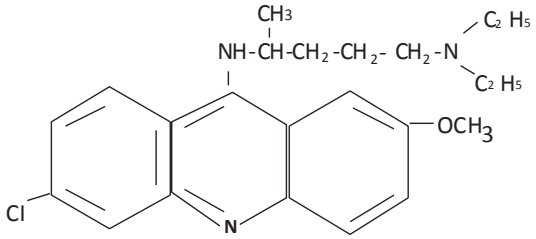
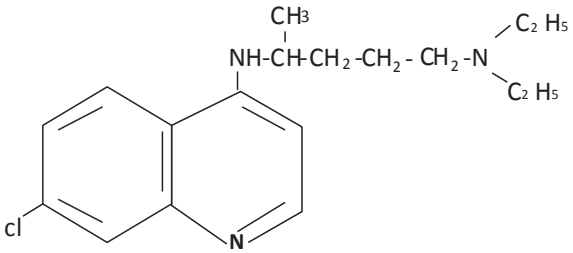
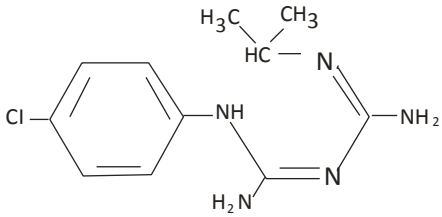
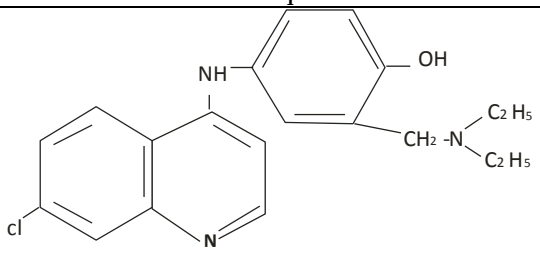
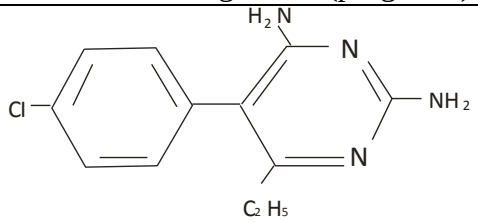
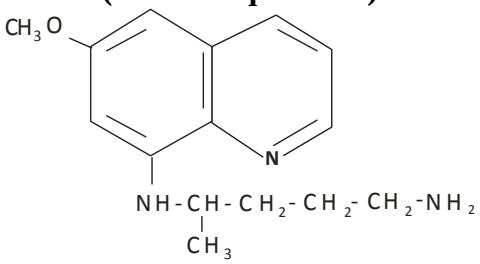
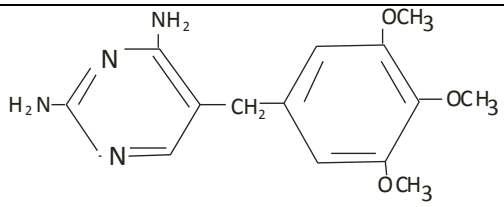
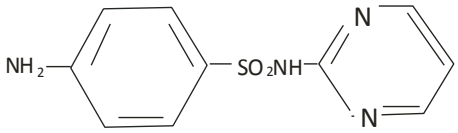
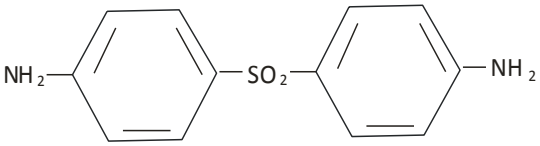
QUINOLINE RING 	**ACRIDINE RING** 
 Quinine	 Quinacrine
(4-Aminoquinolines)  Chloroquine	**(Dihydrofolate reductase inhibitors)**  Chloroguanide (proguanil)
 Aminodiaquine	 Pyrimethamine
(8- Aminoquinoline)  Primaquine	 Trimethoprim

Table 3: Structural Formulas of Antimalarial Drugs (Continued)

Sulfones and Sulfonamides	
 Sulfadiazine	 Dapsone (diaminodiphenylsulfone, DDS)

2.3 TREATMENT OF MALARIA USING HERBAL MEDICINE

Quinine was the first known antimalarial. It is a 4-quinolinemethanol derivative bearing a substituted quinuoline ring. The use of quinine in Europe began in the 17th century, after the Incas of peru informed the Spanish Jesuits of the antimalarial propereties of the bark of an evergreen mountain tree they called quinquina [later called Cinchona after Francisco thenriquez de Ribera (1576-1639), countess of Chinchona and wife of the Peruvian Viceroy] the bark, when made into an aqueous solution, was capable of curing most forms of malaria. It was listed in the London pharmacopeia of 1677. The alkaloid (quinine) derived from it was isolated in the mid-1820s. Quinine, a very bitter substance, has been used by millions of malaria sufferers. Recently it has been employed successfully to treat chloroquine-resistant strains of *P. falciparum* and is considered the drug of choice for these resistant strains (Lemke and Williams, 2012).

A second class of chemicals that played a role in the development of synthetic antimalarials was the 9-aminoacridines. 9-Aminocridine was known to exhibit antibacterial activity, whereas a derivative of 9-aminoacridine (quinacrine) synthesized in 1934, was found to possess weak anti-malarial activity. With the beginning of World War II and concern about interruption of the supply of Cinchona bark from the East Indies, a massive effort was begun to search for synthetic alternatives to quinine and the development of more effective antimalaria agents than quinacrine. With a basic understanding of the structure-activity-relationship of quinine and the chemical similarities seen with quinacrine, it is easy to visualize the relationship between these agents and the synthetic antimalarials. The 4-aminoquinoline, chloroquine and hydroxychloroquine are structurally similar to the right half of quinacrine, the 8-aminoquinoline, pamaquine and primaquine; retain the methoxyquinoline nucleus of quinine and quinacrine. The quinoline -4-

methanols, mefloquine and halofantrine show similarity to the 4-quinolinemethanol portion of quinine (Lemke and Williams, 2012).

An increasing reliance on the use of medical plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants (Okoli and Iroegbu, 2005). The use of medicines from plants in the form of local medicine had been an age-long practice and the medicinal values of these plants may be due to the presence of minute doses of bioactive principals called phytochemicals (Omojate, 2012).

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people. Nowadays, it has been accepted that infectious disease are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries (Iwu *et al.*, 1999). In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents. The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day used herbal medicines (Erdemeier *et al.*, 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as source of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to have been brought under control. There are global problems of multiple antimicrobial resistance (Adebayo, 2001; Aguiyi, 2006); as well as the emergence of new disease and the resurrection of previously eradicated diseases. Most of the current antimicrobial drugs simply reduce the level of growth of bacteria (bacteriostatic) and some of them are very toxic to the hepatic, renal, haematopoietic and the central nervous systems

(Timothy *et al.*, 2011; Timothy *et al.,* 2012). Antimicrobial resistance among enteric pathogens is becoming a matter of serious concern (Fagbohun *et al.,* 2010) and poses a great threat to global population. Moreso, new microbial strains are being continuously discovered, which are refractory to the current arsenal of drugs (Farnsworth, 1982). This is because antimicrobial resistance leads to therapeutic failures of empiric therapy (Carvalho *et al*., 1988). As a result, it has become imperative to combat the emerging and re-emerging infectious diseases with a view to discovering and inventing new agents of greater therapeutics profile to mitigate frequent outbreaks of disease which has posed a new threat to the global health security (Odetola and Akojenu, 2000).

Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis (Odugbemi, 2008).

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the health care system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use (Inamul, 2004). Recognition and development of medicinal and economic benefits of these

plants are on the increase in both developing and industrialized nations (Sofowora, 1993). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some case) and the cultural and spiritual point of view of the people of these countries (Orisakwe *et al.,* 1997, Omonkhelin *et al.*, 2007).

In developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any anti-infective is limited (Satyajji and Lutfun, 2007). Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase to 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of anti-infectives. In addition, many people are interested in having more autonomy over their medical care. All these make the knowledge of chemical, biological and therapeutic activities of medicinal plants use become necessary (Fagbohun *et al.,* 2010). Much of the exploration and utilization of natural products as antimicrobials arise from microbial sources (Ajaiyeoba *et al.,* 1998). It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin (Ajaiyeoba, 2000). Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifugicidal properties of Lichens, the antimicrobial action of *allinine* in *Allium sativum* (garlic), or the antimicrobial action of berberines in goldenseal (*Hydrastis Canadensis*) (Akande *et al.,* 2011). Plant based antimicrobial represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobial needs more exploration. Plants based antimicrobials have enormous therapeutic

potential (Ajaiyeoba, 2000). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Awe and Omojasola, 2008). They are effective, yet gentle. Many plants have tropisms to specific organs and often act beyond the symptomatic treatment of diseases. An example of this is *Hydrastis Canadensis*. Hydrastis not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds (Murray *et al.,* 2012; Appidi *et al*., 2008).

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed and encouraged. Modern pharmacopoeia still contains at least 25% of drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fueled by the rising cost of prescription drugs in the maintenance of personal health and well being and the bio-prospecting of new plant-derived drugs. The ongoing growing recognition of medicinal plant is due to several reasons, including escalating faith in herbal medicine (Azu *et al.,* 2011).

Africa is endowed with many plants that can be used for medicinal purposes to which they have taken full advantage. In fact, out of the approximated 6400 plant species used in tropical Africa, more than 4000 are used as medicinal plants. Medicinal plants are used in the treatments of many diseases and illness, the uses and effects of which are of growing interest to Western societies. Not only are plants used and chosen for their healing abilities, but they also often have symbolic and spiritual significance. For example, leaves, seeds, and twigs that are white, black and red are seen as especially symbolic or magical and possess special properties (Ajayeoba, 2000).

Africa plants have long been the source of important products with nutritional and therapeutic value. Coffee originates from Ethiopia, *Strophanthus* species are strong arrow poisons and supply cardenolides for use against cardiac insufficiency, the *Catharanthus roseus* alkaloids are well-known anti-leukaemic agents-just to mention a few examples. Research is continuing on the vegetable material from this continent in an endeavour to find new compounds of therapeutic interests.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine board to license and regulate the practice of herbal practitioners under the supervision of ministries of health. Clearly, it is evident that almost all traditional practice all over the globe indicated herb as an important aspect in the treatment of disease. The importance of plant in the present day method of treatment cannot be over emphasized in developing countries; thousands of rural communities still depend mainly on folklore medicine to cure diseases (Akande *et al.*, 2011). No surprise that as at today plant still forms one of the major sources of medicines used in clinics, generating about 50% medicinal compounds used by pharmaceutical industry, 25% of prescription drugs are derived from tropical plants three quarter of which from folkloric medicines (Inamul; 2004). Such drugs are *Digitalis* used as important drugs for the management of heart failure from *Digitalis purpurea*, Quinine used for treatment of cerebral malaria from *Cinchona* bark etc. Undoubtedly, a lot of medicine have been isolated from plant that are employed in the health sector today even in the possibility of synthetic chemicals serving as drug, plants still hold many species (Evans, 2008).

Today focus is changing and people are drifting from the use of conventional therapy to the use of natural products. Based on World Health Organization (WHO) report, some 3.4 billion people

in the developing world depend on the plant based traditional medicines (Satyajii and Lutfun, 2007). So also according to WHO, 80% of the world populations rely chiefly on plant based traditional medicines especially for their primary health care needs. About 60 million people are estimated to use herbal remedies each year affording cost of about 3.2 billion Dollars in USA, $6billion in Europe, more than $2 billion in Germany, over 2.3 billion Dollars in China, $ 2.1 billion in Japan, and $1-2 billion in Malaysia etc. (Inamul, 2004).

Though in Nigeria the statistics are not documented, it is clear that huge amount of money is being spent on herbal traditional medicine evidenced by ever increasing number of such products and their demands. Among the uses of herbal therapy is in the treatment of infective disease which form a high percentage of the diseases affecting man all over the world today. The results presently arising from the use of available chemotherapeutic agents are even encouraging factors to the use of herbs. This becomes more serious especially with the claim of benefits of herbal medicines over synthetic counterpart. People seem to have understood and chose to avoid the debilitating side effects that come along with some synthetic chemicals. This coupled with the incidence of resistance to most of the existing chemotherapic agents by microorganisms; re-establish the strong need for anti-infective from natural sources. Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern (Fagbohun *et al.*, 2010). There is need for the development of new antimicrobial; due to acquired resistance more importantly, from natural sources as this delays resistance (Ajaiyeoba, 2000). Today, there is need to study plants to properly establish those whose efficacy have been a claim (Evans, 2008).

In Nigeria many herbs/plants are used in the treatment of diseases some of which are of microbial origin.

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials (Farmsworth and Moris, 1976).

Herbal medicines can be formulated into:

1. Herbal tea, are the resultant liquids of extracting herbs into water.
2. Decoctions are long term boiled extract usually of a harder substance like roots or bark.
3. Maceration is the old infusion of plants with high mucilage – contents such as sage, thyme etc.
4. Tinctures are alcoholic extract of herbs, usually obtained by combing 100% pure ethanol or (a mixture of 100% ethanol with water with the herbs). A completed tincture has an ethanol percentage of at least 25% (sometime up to 90%)
5. Herbal wine and elixirs are alcoholic extracts of herbs, usually with an ethanol percentage of 12-38%. Herbal wine is a maceration of herbs and spirits.
6. Syrups are extracts of herbs made with syrups or honey. Sixty five parts of the sugar are mixed with thirty five parts of water and herbs. The whole is then macerated for three weeks. (Geert Verhelst).

2.3.1 Benefits of Using Herbal Medicines

- They cost less: the herbal madicine has relatively lesser cost to conventional medicine.
- They may have fewer side effects
- There is choice on how to use them: they can be used in variety of ways. Depending on the kind of herb that is to be used. Some herbs can be made into tea, capsule or tablet form.

- They can be used for more than one condition; most conventional drugs are designed for one specific health problem, whereas many herbal machines act on several parts of the body at once.

2.4 PHARMACOGNOSTIC STANDARDIZATION OF HERBAL MEDICINES

Standardization of drugs is the process of establishing or prescribing a set of peculiar identities, specific characteristics which are generally unique and of ushered qualities. Pharmacognostic standardization of drugs is a series of laboratory experiment which reveal and assemble a set of inherent peculiar characteristics such as, constant parameter, definite, qualitative and quantitative values or specific and unique features on the basis of which similar herbal medicine, claimed to be the same, can be compared for the purpose of authenticity, efficacy, genuiness, purity, reproducibility, and overall quality assurance. Uniformity of quality is promoted by the use of standard which numerical qualities by which the quality of commodities may be assessed (Inya-Agha, 2006). Specific standards obtained through experimentation may be compiled into a monograph of the particular medicinal plant. Monograph of selected medicinal plant can be assembled together to constitute a herbal pharmacopoeia. Herbal pharmacopoeia essentially provides parameter for any national drug regulatory requirements or dossiers. Pharmacognostical standardization of the herbal medicine is the provision of the standard official monograph to include the essential characteristics of the plant components. This is done for the purpose of correct identification and qualification such that any other sample of the plant at any other time can be compared with and related to the original sample well subjected to recommended monograph. Such other samples can either be accepted (if found to comply with monograph) or rejected if (grossly below the standard). Beside the efficacy and safety, the provision of

pharmacognostical standard is a free pre-requisite for herbal formulation and clinical exploitation (Inya-Agha, 2006).

2.5 NIGERIAN MEDICINAL PLANTS AS A SOURCE OF ANTIMALARIAL COMPOUNDS

Nigeria has rich flora diversity and many of the plant species are used by some indigenous people for medicinal purposes. Some plant species are used for malaria treatment across all ethnic and cultural groups in the country (Adebayo and Krettli, 2011). Plants used in Nigeria for treatment of malaria include *Morinda lucida, Khaya senegalensis, Khaya grandifoliola, Quassia amara, Quassia undulate, Enantia Chlorantha, Carica papaya, Fagara zanthoxyloides, Spathodea campanulata, Alstonia boonei*, and *Azadirachta indica* (Adebayo and Krettli, 2011; Obih and Makinde, 1985; Isah *et al*., 2003; Phillipson and Wright, 1991).

Several purified compounds from plants used in Nigerian folk medicine have been isolated and evaluated for their antimalarial activity. Among the active compounds isolated majority are alkaloids, followed by limonoids (Adebayo and Krettli, 2011). Some of the alkaloids isolated are: fagaronine, a benzophenanthridine alkaloid derived from the root extract of *Fagara zanthoxyloides* (Kassim *et al*., 2005); palmatine and jatrorrhizine, protoberberine alkaloids isolated from *Enantia chlorantha* (Vennerstrom and Klayman, 1988); akuammiline, akuammidine, akuammine, akuammigine, akuammicine, picraline, and alstonine isolated from *Picralima nitida* (Ansa-Asamoah *et al*., 1990); gedunin and meldenin from *Azadirachta indica* (MacKinnon *et al*., 1997); methylangolensate, 7-deacetylkhivorin, 1-deacetylkhivorin, and 6-acetylswietenolide and gedunin isolated from *Khaya grandifoliola* (Bickii *et al*., 2000); fissinolide and its 2,6-dihydroxy derivative from *Khaya senegalensis* (Khalid *et al*., 1998).

Other phytochemicals with antiplasmodial activities in Nigerian folk medicinal plants are: azadirachtin (a tetranortriterpenoid) from *Azadirachta indica* (Butterworth and Morgan, 1968); ursolic acid isolated from the stem bark of *Spathodea campanulata* (Amusan *et al*., 1996); anthraquinones such as damnacanthal from *Morinda lucida* (Koumaglo *et al*., 1992; Sittie *et al*., 1999); sesquiterpene lactone, Tagitinin C, present in the leaves of *Tithonia diversifolia* (Goffin *et al*., 2002); Simalikalactone D from *Quassia amara* leaves (Bertani *et al*., 2006).

In this present study, nine Nigerian medicinal plants (*Kigelia africana, Baphia pubescens, Morinda lucida, Synclisa scabrida and Buchholzia coriaceae, Rauwolfia vomitoria, Nauclea latifolia, Anthocleista djalonensis and Moringa oleiferae*) were selected as candidates for study of their antimalarial properties.

2.6 *KIGELIA AFRICANA*

2.6.1 Taxononmy

Family: Bignoniaceae

Genus: *Kigelia*

Species: K*igelia africana (*Lam; Benth)

Synonyms: *Bignonia africana* Lam. (basionym); *Tecoma africana* (Lam.) G.Don; *Crescentia pinnata* Jacq; *Kigelia pinnata* (Jacq) DC, *Kigelia abyssinica* A. Rich; *Kigelia aethiopica* Decne.

Common names: The genus Kigelia has one species and occurs only in Africa (Dalziel, 1956). The genus name comes from the Mozambican Bantu name, Kigelia – keia, while the common names sausage tree and cucumber tree refer to the long, sausage-like fruit. Its name in Afrikaans ‘worsboom’ also means sausage tree, and its Arabic name means”the father of kit bags”.

Other vernacular names are Worsboom (Afrikaans), Abu shutor, Abu sidra, Um mashatur, Um shutur (Arabic), Saucissonnier (French), Leberwurstbaum (German), Arbol De Las Salchichas (Spanish), and Ogilisa ofia / Utulubenyi (Igbo).

2.6.2 Uses of *K. africana*

The plant is used as an abortifacient. It is used for the treatment of the side effects of parturition (childbirth), relieves an inflamed spleen (splenitis) and contains anti-inflammatory and anti microbial properties. Traditional alcohol (muratina) made from its fruit extract is used to bath children for treatment of measles. Either its crushed dried fruit or its fresh fruits are used in ethnomedicine to treat ulcers, sores, and syphilis. Its fruit contain antibacterial properties. A decoction made from its roots is drunk to treat gastro-intestinal problems. Its leaf extract is applied onto wounds, to treat epilepsy and venereal diseases. A decoction made from its leaf is drunk to treat malaria. A juice extract from its fruit is used for treating wound. A mixture made from mixing ash harnessed from roasting its leaf together with honey is used in treating high blood pressure. In the cosmetic industry its fruit' extract are used in making beauty, anti skin aging and skin ointment products that are used against eczema and psoriasis due to its anti microbial properties. Its roots are used in the dye or colourant industry to make related products due to its bright colour extracts. Its fruit is traditionally used to make traditional beer commonly known as "muratina" by the Kikuyu people of Kenya. Its fresh fruit is poisonous and cannot be directly eaten. The mode of propagation is by seed (Anowi *et al.*, 2014).

Figure 3: ***Kigelia africana***
https://www.123rf.com/photo_21623307_sausage-tree-fruit-kigelia-africana-.html (Date accessed: 27.08.2018)

2.7 *BAPHIA PUBESCENS*

2.7.1 Taxonomy

Taxon: *Baphia pubescens* Hook. F

Family: Leguminosae –papillomoideae

Genus: *Baphia*

Species: *pubescens*

Synonym: *Baphia bancoensis* Aubrev

Common names: Abosi ofia (Ibo)

English names: Benin camwood

Other names: Awewi, Urohun, Maajigi

Figure 4: ***Baphia pubescens***
http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=5972 (Date accessed: 27.08.2018)

2.7.2 Medicinal uses of *B. pubescens*

The leaf or leaf juice is applied against parasitic skin diseases. A leaf infusion is drunk to cure enteritis and other gastrointestinal problems (FAO, 1996).

In Ghana, Cote d'Ivoire and Nigeria the leaves and bark are considered haemostatic and anti-inflammatory, and are used for healing sores and wounds (Akande *et al* 2001). In Cote d' Ivoire powdered leaves are taken with palm wine or food to cure venereal diseases, and leaf sap is applied as eye drops against jaundice. An extract of young leaves with some salt and red pepper is used as nose drops against headache.

In Nigeria powdered heartwood is made into an ointment with shea butter (obtained from the seeds of *Vitellaria paradoxa* C.F. Gaertn.) which is applied against stiff and swollen joints, sprains and rheumatic complaints. In Sierra Leone, a bark decoction is drunk to cure cardinal pain and bark and leaves are prepared as an enema to treat constipation (Kumar *et al* 2008).

In Nigeria and Ghana, the pounded dried root mixed with water and oil, is applied to a ringworm-like fungus attack. In Cote d' Ivoire a leaf extract of camwood and *Senna occidentalis* L. Link is drunk against asthma. In Benin a decoction of the leaves is taken against jaundice and diabetes. In combination with leaves of *Morinda lucida*, it is used to treat female sterility and painful menstruation. An ointment made from the leaves showed anti-inflammatory activity in mice. Extracts of fresh leaves of *B. pubescens* inhibited digestion in mice and rats, and showed anti-diarrhea properties (Fredrick *et al.,* 2014).

2.8 *BUCHHOLZIA CORIACEA*

Buchholzia coriacea was named after R. W. Buchholz who collected the plant in Cameroon in the late 19th Century. It belongs to the family capparacceae (Keay, 1989). The seed of *B. coriacea* has medicinal values. These seeds gave the plant a common name wonderful kolanut because of its usage in traditional medicine. The seeds are covered in purple aril which are chewed in Ivory Coast and has a pungent taste (Nwaehujor *et al.*, 2012).

2.8.1 Taxonomy

Family: Capparaceae Juss

Genus: *Buchholzia*

Specie: *coriacea*

Vernacular names of *B. coriacea* are Cola pimento, elephant cola, oignon de Gorille and Okpokolo in Igbo (Palombo, 2006).

Figure 5: ***Buchholzia coriacea***
http://forestcenter.iita.org/index.php/manual-page/buchholzia-coriacea-engl/ (Date accessed: 27.08.2018)

2.8.2 Uses of *B. coriacea*

The bark can be made into a pulp for inhalation or into a snuff to relieve headache, sinusitis, and nasal congestion in Ivory Coast; smallpox or skin itching in Gabon. The pulped bark is applied to the chest to treat chest pains and boils. In Liberia, the seeds are used on skin eruption and internally for worms. In Ivory Coast, the crushed up seeds, are pasted over the stomach for difficult childbirth. It is also considered anthelmintic (worm expeller). It is used as cough medicine, and in the treatment of ulcer. It is also used in the treatment of hypertension by drinking the fluid squeezed out of the leaves with pea leaves and small salt. Plants that belong to the botanical family Capparidaceae have been used for the treatment of syphilis, dressing of wounds, chronic ulcers, gonorrhea, convulsion in children, anthelmintics, as aphrodisiacs, and for the treatment of snake bites. In the Ivory Coast the twig bark decoction of the plant *B. coriacea* is used for the treatment of rheumatism and kidney pain, it is also used for the treatment of pain in the back (fruit pulp massaged in). For the treatment of earache, seeds are pounded in a little bit of water and the resulting liquid is dropped into the ear. The Ebri tribes bathe smallpox victims with the bark decoction of the plant *B. coriacea* (Anowi *et al.,* 2012).

Young leaves of the plant *B. coriacea* are used in a gruel poultice for ulcers and boils. In Gabon pounded bark of the plant *B. coriacea is* used as a lotion against scabies, the fruit of the plant *B. coriacea* as an anthelmintic. In former times young warriors were given fresh roots of the plant *B. coriacea* to stimulate them before battle. The seeds or kernels of the plant *B. coriacea* are edible; they have a spicy taste and can be used as a condiment (spice). The ground seeds or kernels of the plant *B. coriacea* are a component of a traditional and valued aphrodisiac or stimulant that is sold on local markets in Africa (Cameroon). The Africa plant *B. coriacea* is used as stimulant, tonic, and aphrodisiac (Anowi *et al.,* 2012).

2.9 *SYNCLISIA SCABRIDA*

2.9.1 Taxonomy

Family: Menispermaceae

Genus: *Synclisia*

Species: *scabrida* Miers

2.9.2 Ethnobotanical uses of *S. scabrida*

In Nigeria and Cameroon an alcoholic leaf decoction is drunk to treat gastric ulcers. In Nigeria the root soaked in alcohol or macerated in boiling water is taken to treat malaria, to prevent threatened abortion and as a common medicine to calm patients with mental disorders e.g. psychoses. In Gabon the bitter rooty, sometimes mixed with stem bark of *Garcinia klainii* Pierre ex Engl., is put in palm wine and drunk to treat venereal disease and as an aphrodisiac and also to treat prostate problems, asthma and hernia. In Congo pregnant women may tie a piece of plant as liana around the waist to avoid spontaneous abortion. In Gabon a root decoction is used in trial by ordeal ceremonies: when it causes constipation one is innocent, when it causes diarrhea, one is guilty. The root bark contains a yellow dye of unrecorded use. The leaves are used as protein – rich folder for ruminants (Schmelzer *et al.,* 2008; Obi *et al.,* 2000; Ohiri *et al.,* 1989; Okoli and Iroegbu, 2005; Orisakwe *et al*., 1996).

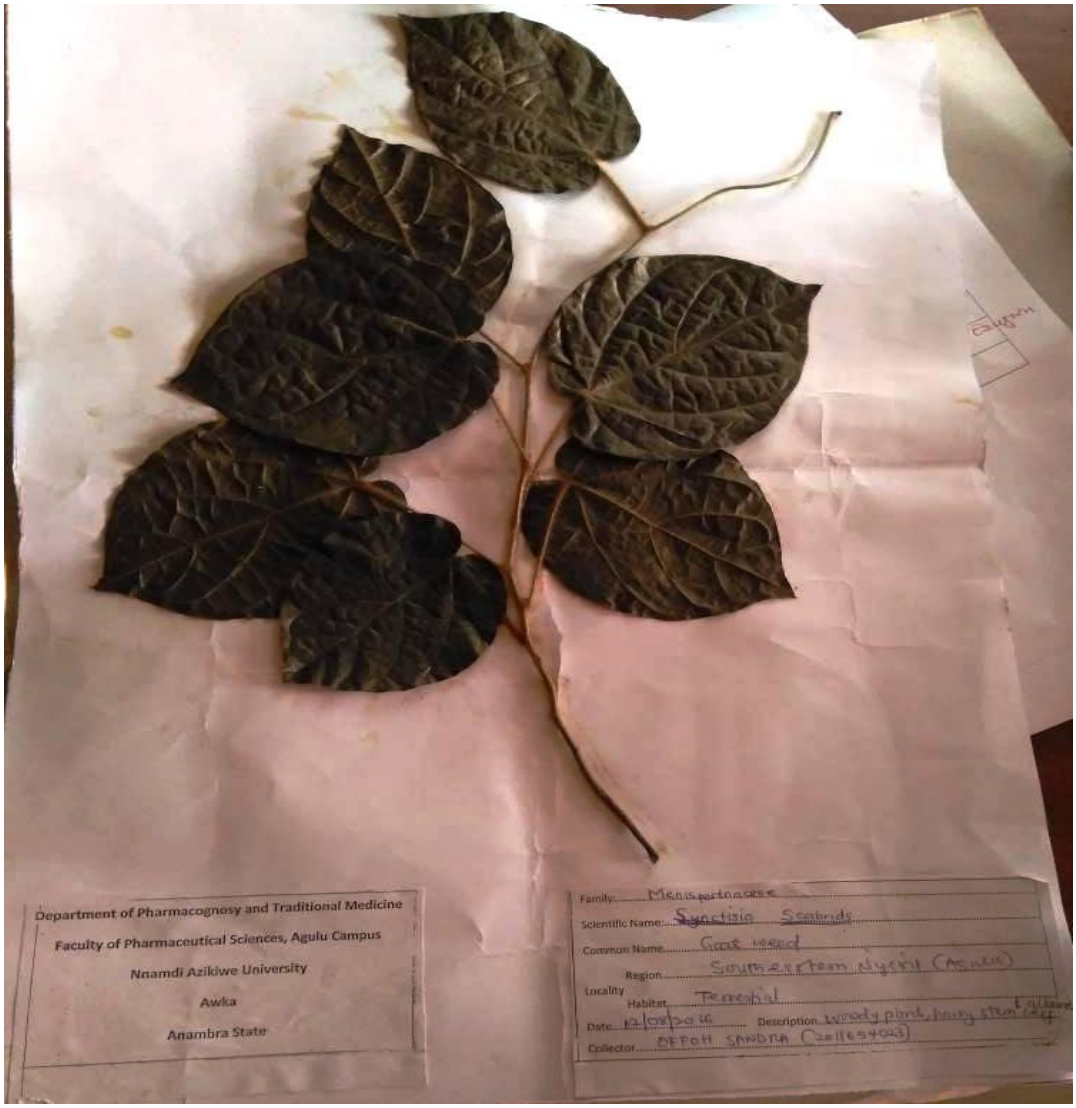


Figure 6: ***Synclisia scabrida***

2.10 *ANTHOCLEISTA DJALONENSIS*

2.10.1 Taxonomy

Family: Loganiaceae

Genus: *Anthocleista*

Specie: *djalonensis*

Common name: Cabbage tree

2.10.2 Geographical distribution of *A. djalonensis*

A. djalonensis occurs from Guinea Bissau east to Cameroon. It occurs in rather dry localities, in savannah or thickets, from sea-level up to 500 m altitude. In Ghana, *A. djalonensis* flowers in April and May, and in Nigeria from March to May. Fruits occur in Nigeria in October and November (Schmelzer et al., 2008).

Figure 7: ***Anthocleista djalonensis***
http://picssr.com/tags/anthocleista (Date accessed: 27.08.2018)

2.10.3 Traditional Uses of *A. djalonensis*

Traditionally, *A. djalonensis* is used to treat wound, malaria, constipation, dysentery, diarrhoea, hepatitis, skin infection, and inflammation (Okoli and Iroegbu, 2004; Aiyeloja and Bello, 2006).

In Mali, root maceration is taken alone or with honey to treat malaria and a root decoction or root powder in porridge is taken to treat abdominal pain. A root decoction is also taken to treat hernia of the groin. In southern Nigeria a decoction of the roots with potash is taken to treat fungal skin infections and filarial worm infections, including Loa Loa, filariasis. A tea made from the chopped soft root bark soaked in water is taken to treat thrush. An alcoholic leaf extract is taken to treat diarrhoea and dysentery. In Sierra Leone a decoction of dry fallen leaves is drunk to treat jaundice. In Guinea Bissau a bark infusion is used to treat broken bones in women. In Côte d'Ivoire the Attié people use an extract of twig bark as eye drops to treat diarrhoea in babies. Powdered stem bark mixed with the roots of *Aloe* is taken to treat hepatitis, jaundice and cirrhosis (PROTA, 2008).

2.11 *MORINDA LUCIDA*

Amongst the medicinal plants commonly use in Nigeria for management/ treatment of various types of ailments is *Morinda lucida* Benth. (L.) (*Rubiaceae*). It is a tropical West African rainforest plant commonly known as Brimstone tree. *M. lucida* is a medium size tree about 15m tall with scaly grey bark, short crooked branches and shining foliage. The leaves are used as "oral teas", which are usually taken orally for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infections. The leaves have also been reported to possess strong trypanocidal and aortic vasorelaxant activities (Asuzu and Chineme, 1990; Adeleye *et al.*, 2018). Further studies have shown that leaf and stem bark of *M. lucida* possess

anticancer, hepatoprotective, cytotoxic and genotoxic, antispermatogenic, hypoglycemia and antidiabetic activities (Daziel, 1973). The major constituents of *M. lucida* extract are the various types of alkaloids, anthraquinones, and anthraquinols. Ten anthraquinones have been isolated and characterized from the stem of the plant.

2.11.1 Taxonomy

Morinda lucida comprises approximately 80 species, distributed in all tropical regions of the world.

Family: Rubiaeae

Genus: *Morinda*

Species: *lucida*

Taxon: *Morinda lucida* Benth

Common Names: Brimstone tree

Igbo Names: Akpakwulu nniewu

Figure 8: ***Morinda lucida***

2.11.2 Ecology of *M. lucida*

M. lucida grows in grassland, exposed hillsides, thickets, forests, often on termite mounds, sometimes in areas which are regularly flooded, from sea-level up to 1300 m altitude.

2.11.3 Ethnomedicinal uses of *M. lucida*

M. lucida is a native plant growing in many African countries and widely used as a medicine in West Africa. It is generally used as ingredients of fever teas, which are usually taken, for the traditional treatment of malaria. In West Africa, *M. lucida* is an important plant in traditional medicine. In Nigeria, *M. lucida* is used in the preparation of traditional medicines against fever. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. In some cases, the plant is employed in the treatment of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhea (Adesida *et al.*, 1972). In Cote d' Ivoire a bark or leaf decoction is applied against jaundice and in Democratic Republic of Congo, the decoction of the stem bark or leaf is combined with a dressing of powdered root bark against itch and ringworm (Abbiw, 1990).

However, *M. lucida* is used generally for febrifuge, analgesic and laxative while the decoction of the stem bark is used for the treatment of severe jaundice. *M. lucida* is used locally in the treatment of irregular menstruation, insomnia and jaundice (Lawal *et al.*, 2012). M. *lucida* is also used in the treatment of wound infections, abscesses and chancre (Burkill, 1991). Also amongst the Igede people in Benue State, Nigeria, the decoction of the *M. lucida* is used twice or thrice daily as anti-diarrhea, while the leaves are used for treatment of infertility in women (Lawal *et al.*, 2012).

2.12 *MORINGA OLEIFERA*

M. oleifera (Lam.) is a fast-growing, deciduous tree. It can reach a height of 10–12 m (32-40 ft) and the trunk can reach a diameter of 45 cm (1.5 ft). The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up feathery foliage of tripinnate leaves.

2.12.1 Taxonomy

Family: Moringaceae

Genus: Moringa

Species: *Moringa oleifera*

Common names: Drumstick tree, Horseradish tree.

2.12.2 Uses of *Moringa oleifera*

Many parts of the moringa are edible. Regional uses of the moringa as food vary widely, and include: The immature seed pods, called "drumsticks", are popular in Asia and Africa. Leaves are eaten, particularly in Cambodia, the Philippines, South India, Sri Lanka, and Africa. In some regions, the young seed pods are most commonly eaten, while in others, the leaves are the most commonly used part of the plant. The flowers are edible when cooked and are said to taste like mushrooms. The bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries. In Jamaica, the sap is used for a blue dye (Adegoke *et al.*, 2016).

Figure 9: ***Moringa oleifera***
http://drfarrah.online/malunggay-moringa-oleifera-gift-world/ (Date accessed: 27.08.2018)

2.13 *NAUCLEA LATIFOLIA*

2.13.1 Taxonomy

Family: Rubiaceae-A.L.de jussieu, 178

Genus: *Nauclea*

Species: *latifolia*

Botanial name: *Nauclea latifolia*

Common name: African peach

Igbo name: Mbilinu or Ubilinu

2.13.2 Traditional medicinal uses of *N. latifolia*

N. latifolia is used in the treatment of diabetes; the root is febrifuge and tonic. It is used in the treatment of fevers, indigestion; the fruit is eaten as a cure for coughs. The bark and roots of the plant contain more than one percent of an opioid that is clinically identical to the analgesic drug tramadol. *N. latifolia* has been reported to be used by traditional healers to arrest pre-term labor (Nworgu *et al.,* 2010). The decoction of the leaves is recommended for stomach upset in children; the infusion of the root is also used as a remedy for stomach upset in adult (Gills, 1992).

Figure 10: ***Nauclea latifolia***
http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=2015 (Date accessed: 27.08.2018)

2.14 *RAUVOLFIA VOMITORIA*

2.14.1 Taxonomy

Family:	Apocynanceae
Genus:	Rauvolfia
Species:	*vomitoria*
Binomial name:	*Rauvolfia vomitoria* Afzel
Synonyms:	*Hylucium owariense* Afzel; *Rauvolfia senegambiae* A. DC; *Rauvolfia caffra*; *Rauvolfia mombasinna*; *Rauvolfia oreogiton*; *Rauvolfia obscura*
Common Names:	English: Swizzle Stick, serpent wood, serpent shake root; Yoruba: asofeyeje, ira, iran-igbo; Igbo: akanta; Hausa: wada; Bini: Akata; Efiki: utoenyin

2.14.2 Ecology and distribution

R. vomitoria occurs naturally in gallery forests but is mostly found in forest re-growth where fallow periods are prolonged. *R. vomitoria* is associated with palms *Trema guineensis* and *Combretum spp* and is one of the last species to disappear in the particular seral stage. *R. vomitoria* is considered endangered. The plant is native to Cameroon, Democratic of Congo, Ghana, Liberia, Nigeria, Senegal, Sudan, and Uganda.

Figure 11: ***Rauvolfia vomitoria***
https://www.ebay.co.uk/p/15-Seeds-Rauwolfia-Vomitoria-Rauvolfia-Poison-Devils-pepper/22014714224 (Date accessed: 27.08.2018)

2.14.3 Medicine uses of *R. vomitoria*

R. vomitoria is used medicinally in many African countries. The plant is used by Nigerian traditional healers to treat psychiatric patients. The root of *R. vomitoria* administered orally at doses of 400, 600 or 80 0mg/day showed anti-psychotic effect. *R. vomitoria* root extract has curative properties. The bark has purgative and emetic properties. The root extract has arbortifacient property. In Ghana and Nigeria, it is used as emetic and purgative. Rauvolfia is used traditionally against snake bites, lesions, and children are treated with this plant for cerebral cramps, jaundice and gastrointestinal disorders. Watery solution of the bark of *R. vomitoria* is used against such parasites as lice and scabies. In Mali, the roots of *Rauvolfia* are used to treat hemorrhoids and hepatomegaly. It is also used in Mali as sedative for mentally ill persons, and is good for treating tetanus and epilepsy. The pygmies of Congo Basin administer *Rauvolfia* species together with traditional ash salt against diarrhea and with red palm oil against elephantiasis of the legs. It is used as an abortifacient because it contracts the uterus after administration (Okon *et al.,* 2017). Reports showed that the roots of *Rauvolfia* is good for the treatment of snakes bites; insect stings; nevours disorders; intractable skin disorders such as psoriasis, excessive sweating, itching; hypertention; uterine contration in child birth and gynaecological ointment for the treatment of menopausal-disorder. *R. vomitoria* root bark extract is a sedative, hypnotic and good for reducing blood pressure. *Rauvolfia* is good for treating insanity, anti-anxiety agent stimulant to central nervous system. The root is a good anthelminitic and an antidote to snake venom. Its decotion could be given during labour pains to increase uterine contraction. Juice of the leaves of the *R. vomitoria* root bark extract is used for the treatment of corneal opacity of the eyes (Kutalek and Prinz, 2007; Okon *et al.,* 2017).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Chemicals and Reagents

Dragendoff's reagent, Wagner reagents, Hager's reagents, ammonium chloride solution, Fehling's solution 1&2, sulphuric acid, ferric chloride, lead sub-acetate, olive oil, million's reagent, picric acid, molisch reagent, iodine, 10% Buffered formalin**,**

3.1.2 Solvents

Solvents used in general procedures include diethyl ether, ethyl acetate, ethanol, and methanol. HPLC solvents include: methanol (LiChroSolv HPLC grade, Merck, Germany), dstilled and heavy metal free water [obtained by passing through nano- and ion-exchange filter cells (Barnstead) to yield nanopure water].

3.1.3 Glass wares

Glass slides, test tubes, beakers, measuring cylinders, capillary tubes, etc.

3.1.4 Equipment

Microscope; Counting Chamber**;** Haemocytometer**;** Micro-hematocrit centrifuge SH120**;** rotary evaporator; Electronic weighing balance (NAPCO Precision Instruments JA-410); water bath; thermostat oven (DHG-9023A, PEC MEDICAL USA); UV-spectrophotometer (Model 721); analytical HPLC [the analytical HPLC components include: pump (P580A LPG, Dionex, Germany), autosampler (ASI-100, Dionex), photodiode array detector (UVD 340S, Dionex, Germany), Column oven (STH 585, Dionex, Germany), Column Eurosphere (100-C18; 5 μM; 125 x 4 mm; with integrated pre-column, Germany), software (Chromeleon 6.30)].

3.1.5 Others

Oral cannula**,** Standard Cages**,** Cotton wool and Hand gloves, 5mL hypodermic syringe**,**

Vital top feed (Jos, Nigeria)**,** Dissecting kits**,** EDTA container and Plain Container**,** Micro haematocrit Reader**,** Plasticine, Neubaur, Whatmann Filter papers.

3.1.6 Test animals

Male Wistar rats housed in the animal house, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Anambra State were used in this study. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were kept on 12 h light and dark cycles. The animals were allowed free access to food and water *ad libitum* and were placed under standard Laboratory animal house condition.

3.1.7 Test organisms

Chloroquine sensitive strain of *Plasmodium berghei* (strain ANKA) was obtained from the National Institute for Pharmaceutical Research and Development (NIPRID) Abuja, Nigeria, as cryo-frozen stock of parasitized red blood cells (PRBCs).

3.2 METHODS

3.2.1 COLLECTION AND IDENTIFICATION OF PLANTS

The fresh leaves of nine Nigerian medicinal plants (*K. africana, B. pubescens, M. lucida, S. scabrida, B. coriaceae, R. vomitoria, N. latifolia, A. djalonensis* and *M. oleiferae*) were obtained from Ogidi, Idemili North Local Government Area and identified by Mr Ozioko, a Taxonomist with the Biosource Development and Conservation Program (BDCP), Nsukka, Enugu State, Nigeria. The plants were then authenticated and kept in the herbarium with voucher numbers: *K. africana* (PCG 474/A/035), *B. pubescens* (PCG 474/A/056), *M. lucida* (PCG 474/A/005), *S.*

scabrida (PCG 474/A/045), *B. coriaceae* (PCG 474/A/047), *R. vomitoria*, *N. latifolia* (PCG 474/A/005), *A. djalonensis*(PCG 474/A/046), and *M. oleiferae* (PCG 474/A/037). The leaves were air-dried for 2 weeks in the Pharmacognosy laboratory and milled, for use.

3.2.2 PRELIMINARY ANTI-MALARIAL EVALUATION

- **Parasite infection of experimental animals**

The chloroquine sensitive strain of *Plasmodium berghei* (strain ANKA) was obtained from the National Institute for Pharmaceutical Research and Development (NIPRID) Abuja, Nigeria, as cryo-frozen stock of parasitized red blood cells (PRBCs). The parasites were prepared through two cycles of passage of the PRBCs in rats and mice. Donors with parasitaemia level of 40-50% were sacrificed and blood collected by cardiac puncture into heparinized tubes. The blood was then diluted with phosphate buffered saline (PBS) based on parasitaemia level of each donor and the RBC count of normal mice and rats, such that 1 mL blood contained 5×10^7 parasites. The experimental animals were each treated with 1×10^7 PRBCs by intraperitoneal (ip) injection (Basir *et al*., 2012).

- **Curative Test For Malarial Parasites**

A total of 15 albino mice were selected and were grouped into 5 groups of 3 animals per group. All the animals were infected with *Plasmodium berghei*, a zoonotic *Plasmodium* sp. (approximately 1×10^7 infected red cells) by intra peritoneal route. The animals were then left for 72 h for the infection to establish. After 72 h, the animals were given treatment by single oral administration for four days as follows: Group 1 received 0.5mL of 5% tween 80; Group 2 received 50mg/Kg of quinine; Group 3 received 50mg/Kg of methanol extract; Group 4 received 100mg/Kg of methanol extract; and Group 5 received 200mg/Kg methanol extract.

Thin blood smears from the tail of the mice were examined under a microscope for parasitemia levels. The extracts are said to have a curative effect, if the treated animals shows no parasitemia or have survived at least twice as long as the controls.

- **Determination of Parasitaemia**

The blood from the tail of the infected mice was collected and placed on a clean glass slide placed horizontally on the working bench. The slide and the spreader was held at a suitable angle, pulled back to touch the dropped blood on the slide and spread along it. The film was fixed with methanol and lowered into the already prepared Giemsa stains (1 mL of Giemsa +19 mL of buffer) and allowed to stain for 45 min. The slide was lifted off the stain solution with the aid of forceps, excess stain was washed off, allowed to drain and air dried at room temperature. Then parasitaemia was examined microscopically under oil immersion lens and the parasitized level was determined by counting red blood cells out of 200 red blood cells in a random field of microscope.

$$\%\text{Parasitemia} = [No.of\ infected\ cells \div\ No.of\ RBCs\ counted(200)] \times 100$$

3.2.3 FURTHER INVESTIGATIONS OF *B. PUBESCENS*

3.2.3.1 Extraction and fractionation

A weight of 1500g of the pulverized leaves of *B. pubescens* was macerated in 3000mL of absolute methanol (analytical grade) for 48 h and then filtered with whatman (No1) filter paper. The marc was re-extracted until all the extractable constituents were completely washed out and then filtered. Both filtrates were combined and concentrated at 40^{o}C with the rotary evaporator. The weight of the dried extract was obtained using an electronic weighing balance.

A weight of 500 g of methanol extract of *B. pubescens* was dissolved in 750mL of methanol and equal volume of n-hexane was added to the mixture. The mixture of the two immiscible liquids was poured into a separating funnel using a filter funnel and the separating funnel containing the mixture was shaken gently. The separating funnel was fixed on a rector stand and allowed to stand for a while for the two immiscible liquids to separate completely. The lower methanol extract was decanted and collected into a beaker while the upper layer of n-hexane fraction was also collected into a separate beaker. This process was repeated until there was no change in colour in n-hexane fraction. The n-hexane fraction was evaporated to dryness at 35^0C using rotary evaporator, while the methanol fraction was poured back into the separating funnel and the process was repeated using ethyl acetate and butanol respectively. The weights of the individual fractions were obtained using electronic weighing balance and the percentage yields were calculated.

3.2.3.2 Microscopic examination of powdered leaves

Small quantity of the powdered drug was placed on a slide and few drops of chloral hydrate solution were added to it. The mixture was passed across the flame of a Bunsen burner repeatedly until bubbles occurred and allowed to cool for proper clearing of the sample. Two drops of glycerine were added to the slide as mountant and the slide was covered with cover slip and viewed under the microscope. The microscopic characters (such as cork cells, sclereids, fibres, calcium oxalate crystal etc.) were observed and noted.

3.2.4 QUANTITATIVE PHYTOCHEMICAL ANALYSIS

3.2.4.1 Test for alkaloids

A weight of 5g of powdered *B. pubescens* leaf sample was added into a 250mL beaker and 200mL of 10% acetic acid in ethanol was added. It was covered and allowed to stand for 2h. it was filtered and the extract concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, the precipitate formed was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and expressed as percentage of the alkaloids in the leaves (Harborne, 1973; Sofowora, 1993).

3.2.4.2 Test for saponins

To 20 g of powdered *B. pubescens* leaf sample was placed in a conical flask, 100 mL of 20% ethanol added. This was heated at 55^0C over in a water bath for 4 h with continous stirring, and thereafter, the mixture was filtered. The residue was then extracted with 200 mL of 20% ethanol. The extracts were combined and then concentrated to 40 mL over a water bath at 90^oC. The concentrate was transferred into a 250 mL separating funnel, extracted twice with 20mL diethyl ether and shaken vigorously. The ether layer was discarded while the aqueous layer was retained. To the aqueous layer, 60mL of n-butanol was added. Then the n-butanol extract was washed twice with 10mL of 5% aqueous sodium chloride and the remaining solution was heated on a water bath. After evaporation, it was dried in the oven (40^0C) to a constant weight. Finally, the saponin content was calculated as percentage of the initial weight of the sample taken (Harborne, 1973; Sofowora, 1993).

3.2.4.3 Determination of Tannins

Folin-Denis Spectrophotometric method described by Pearson (1976) was used. A measured weight of each sample (1 g) was dispersed in 10 mL distilled water and agitated. This was left to stand for 30 min at room temperature being shaken every 5 min. At the end of the 30 min, it was centrifuged and the extract got. 2.5 mL of supernatant (extract) was dispersed into a 50 volumetric flask. Similarly, 2.5 mL of standard tannic acid solution was dispersed into a separate 50mL flask. A Folin-Denis reagent was measured into each flask, followed by 2.5mL of saturated Na_2Co_3 solutions. The mixture was diluted to mark in the flask, (50 mL) and incubated for 90 min at room temperature. The absorbance was measured at 250nm in a genway model 6000 electronic spectrophotometer. Readings were taken with the reagent blank to zero; the tannin content was given as follows

%Tannin = An/As x C x 100/W x V/Va

Where An =absorbance of the test sample; As = absorbance of the standard solution; C =concentration of the standard solution; W =weight of sample used; V = total volume of extract; and Va = volume of extract analyzed (Harborne, 1973; Sofowora, 1993).

3.2.4.4 Determination of flavonoids

A weight of 1g of powdered *B. pubescens* leaf sample was extracted with methanol, 20mL of acetone, 2 mL of 25% HCL and 1mL of 0.5 % hexamethylenetetramine was added to 25mL of the extract and refluxed at 56 ^{o}C for 30 min. The extract was filtered and was re-extracted twice with 20 mL of acetone for 10 min. After cooling and filtration, the extract was made up to 100 mL with acetone (basic sample solution), 20 mL of this basic sample solution was mixed with 20 mL of water and then extracted with ethyl acetate (first with 15 mL and then three times with 10 mL). The ethyl acetate extracts were rinsed twice with water then filtered and made up to 50 mL

with ethyl acetate. To 10 mL of this extract, 0.5 mL of 0.5% solution of sodium citrate and 2 mL of $AlCl_3$ (prepared by dissolving 2g of $AlCl_3$ in 100 mL of 5% acetic acid in methanol) was added and was made up to 25 mL with 5% methanol solution of acetic acid (sample solution). The same procedure was performed with blank sample solution but without $AlCl_3$. After 45 min, the yellow solutions were filtered and the absorbance reading at 425 nm evaluated. Then the yield was calculated as quercetin percent using the following expression g% = A x 0.772/b, where A is absorbance and b represents the mass of dry herbal material in grams(Harborne, 1973; Sofowora, 1993).

3.2.4.5 Determination of extractives

- **Alcohol extractive value**

Two (2) grams of the sample was macerated in 100 mL of absolute ethanol for 24 h and filtered. The filtrate was dried in the oven at the temperature of $105^{O}C$ to obtain a dry extract with constant weight. The weight of the dried extract was determined and recorded (Harborne, 1973; Sofowora, 1993).

- **Water extractive value**

A weight of 2g of the sample was macerated in 50 mL of chloroform water in the ratio 1:400 for 24 h and filtered. The filtrate was dried in the oven at the temperature of $105^{O}C$ to obtain a constant weight dry extract. This was cooled in the desiccators. The weight of the dried extract was determined and recorded (Harborne, 1973; Sofowora, 1993).

3.2.4.6 Determination of moisture content

A preheated porcelain crucible was weighed (W_1) and 2g of the powdered sample was measured into the crucible and reweighed (W_2). The sample was gradually heated in the oven up to the temperature of $105^{o}C$ for about 4 h until a constant weight was obtained. The heated sample was

cooled in the desiccators and weighed (W_3) and the moisture content was calculated (Harborne, 1973; Sofowora, 1993).

3.2.4.7 Total ash values

A porcelain crucible was placed in muffle furnace for about 15 min at $35^{O}C$, cooled in a desiccators for about 1 h and the crucible was weighed (W_1). Three grams of the sample is accurately weighed into the preheated porcelain crucible and reweighed (W_2). The sample is ashed in a muffle furnace at $650^{O}C$ for about 6 h until the sample turns grey (white ash). The crucible is removed with crucible tong, cooled in a desiccator and reweighed (W_3). The percentage ash content is determined (Harborne, 1973; Sofowora, 1993).

3.2.4.8 Water soluble ash value

A porcelain crucible was placed in muffle furnace and ignited to a constant weight at the temperature of $450^{O}C$, cooled and weighed (W_1). Two grams of the powdered drug was placed in the crucible and reweighed (W_2). The crucible containing the drug was incinerated at low temperature initially to burn off the carbon content. The heat was gradually increased until all the carbon was burnt off. The crucible was cooled in desiccators, reweighed and the content was transferred into a small beaker. About 5mL of water was added to the content and boiled for 5 min, filtered with an ashless filter paper and the filter paper containing the residue was dried in the oven. The filter paper containing the residue was compressed into the crucible and subjected to heat until the ash less filter paper was eliminated and the crucible is reweighed (Harborne, 1973; Sofowora, 1993).

3.2.4.9 Acid insoluble ash value

The total ash got from incinerating the powdered leaf at 450^{o}C was transferred into a beaker containing 25mL of dilute hydrochloric acid, boiled on a water bath for about 5 min and filtered with an ashless filter paper. The beaker and crucible were washed repeatedly through the filter paper with hot water until they are free from acid (i.e. neutral to litmus paper). The insoluble matter and the ashless filter paper were dried in the oven, ignited in the muffle furnace at 450^{o}C to a constant weight and the amount of acid insoluble ash per gram of the powdered drug was calculated (Harborne, 1973; Sofowora, 1993).

3.2.5 CHROMATOGRAPHIC ANALYSIS

3.2.5.1 Vacuum liquid chromatography

The ethyl acetate fraction which was found to be the most effective fraction against malarial parasites was subjected to vacuum liquid chromatography (VLC) using silica gel 200- 400 mesh size, 400 g, sintered glass 5 L and eluted with 500 mL each of *n*-hex:EtOAc (10:0, 9:1,8:2, 7:3, 6:4, 5:5: 4:6, 3:7, 2:8, 1:9, and 0:10) followed with DCM:MeOH (10:0, 8:2, 5:5, 2:8) and these resulted to 15 pooled fractions.

Table 4: Solvents systems

Samples	Label
D1	*n*-Hex:EtOAc 5:5
D2	DCM:MeOH 2:8
D3	DCM:MeOH 5:5
D4	DCM:MeOH 10:0
D5	*n*-Hex:EtOAc 9:1
D6	*n*-Hex:EtOAc 6:4
D7	*n*-Hex:EtOAc 7:3
D8	*n*-Hex:EtOAc 1:9
D9	DCM:MeOH 8:2
D10	*n*-Hex:EtOAc 8:2
D11	*n*-Hex:EtOAc 3:7
D12	*n*-Hex:EtOAc 2: 8
D13	*n*-Hex:EtOAc 4: 6
D14	*n*-Hex:EtOAc 0:10
D15	EtOAc

3.2.5.2 Sephadex separation

About 183.1, 283.2, 252.8 and 565.9 mg each of ethyl acetate sub-fractions D2, D5, D7 and D10 respectively were subjected to sephadex LH-20 separation. D2 was eluted with methanol while other sub-fractions were eluted with DCM:MeOH (1:1). The flow rate was maintained at 0.4 mL/min. Eluents were collected in 2 mL per fraction up to 80 fractions for D2, 52 fractions for D5, 66 fractions for D7 and 64 fractions for D10. Each fraction was spotted on the TLC plate 1 cm apart from neighboring fraction and 2 cm apart from the base. The samples were drawn up the plate (10 cm) via capillary action using DCM:MeOH in the ratio of 2:8 (D2), *n*-hex:EtOAc 9:1 (D5), 7:3 (D7) and 8:2 (D10) as mobile phases. The plate was allowed to develop in the TLC tank till the solvent was about 1 cm below the top of the plate. The fractions were bulked based on their characteristic colour and movement when visualized with a UV lamp.

Table 5: Batching/Bulking of Similar Fractions

VLC FRACTIONS	SEPHADEX BULKED SUB-FRACTIONS	WEIGHT (mg)
D2 (DCM:MeOH; 2:8)	D2a (1-16)	59.5
	D2b (17-32)	63.1
	D2c (33-64)	20.4
	D2d (65-128)	40.1
D5 (*n*-Hex:EtOAc; 9:1)	D5a (1-14)	52.6
	D5b (15-20)	46.5
	D5c (21-22)	77.4
	D5d (23-34)	28.1
	D5e (35-36)	44
	D5f (37-52)	34.6
D7 (*n*-Hex:EtOAc; 7:3)	D7a (1-38)	100.2
	D7b (38-50)	99.3
	D7c (51-66)	53.3
D10 (*n*-Hex:EtOAc; 8:2)	D10a (1-10)	88.4
	D10b (11-20)	49.2
	D10c (21-26)	59.3
	D10d (27-40)	166.9
	D10e (41-52)	112.5
	D10f (53-64)	89.6

Table 6: Various Subfractions of *B. pubescens* subjected to HPLC-DAD assay

S/NO	NAME	FRACTION	RATIO	BULK SUB FRACTIONS
1.	FRED 1(D10a)	*n*-Hex:EtOAc	8:2	1-10
2.	FRED 2(D10b)	*n*-Hex:EtOAc	8:2	11 - 20
3.	FRED 3(D10c)	*n*-Hex:EtOAc	8:2	21 - 26
4.	FRED 4(D10d)	*n*-Hex:EtOAc	8:2	27 - 40
5.	FRED 5(D10e)	*n*-Hex:EtOAc	8:2	41 - 52
6.	FRED 6(d10f)	*n*-Hex:EtOAc	8:2	53 - 64
7	FRED 7(D7a)	*n*-Hex:EtOAc	7:3	1-38
8.	FRED 8(D5a)	*n*-Hex:EtOAc	9:1	1-14
9.	FRED 9(D5b)	*n*-Hex:EtOAc	9:1	15 - 20
10.	FRED 10(D5c)	*n*-Hex:EtOAc	9:1	21 - 22
11.	FRED 11((D5d)	*n*-Hex:EtOAc	9:1	23 - 34
12.	FRED 12(D5e)	*n*-Hex:EtOAc	9:1	35 - 36
13.	FRED 13(D5f)	*n*-Hex:EtOAc	9:1	37 - 52
14.	FRED 14(D2a)	DCM:MeoH	2:8	1 - 16
15.	FRED 15(D2b)	DCM:MeoH	2:8	17 - 32
16.	FRED 16(D2c)	DCM:MeoH	2:8	33 - 64
17.	FRED 17(D2d)	DCM:MeoH	2:8	65 - 128
18.	FRED 18(D7b)	*n*-Hex:EtOAc	7:3	39 - 50
19.	FRED 19(D7c)	*n*-Hex:EtOAc	7:3	51 - 66

3.2.5.3 High-performance liquid chromatography

HPLC-DAD assay was carried out on the plant fractions as reported by Akpotu *et al.* (2017). Each of the plant fractions (2 mg) was reconstituted with 2 mL of HPLC grade methanol. The mixture was sonicated for 10 min, followed by centrifugation at 3000 rpm for 5 min. Then, 100 μL of the dissolved samples were transferred into HPLC vials containing 500 μL of the HPLC grade methanol. The HPLC-DAD analysis was carried out on the samples with a Dionex P 580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany). Detection was at 235, 254, 280, and 340 nm. The separation column (125 mm × 4 mm; length × internal diameter) was pre-filled with Eurospher-10 C18 (Knauer, Germany) and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. The absorption peaks for each sample were analyzed by comparing with those in the HPLC-ultraviolet (UV)/visible database, which contains over 1600 registered compounds.

3.2.6 TEST FOR CURATIVE ACTIVITY (RANE'S TEST) OF THE VLC FRACTIONS

The schizontocidal activity of the VLC subfractions on established infection was evaluated using the method described by Ryley and Peters (1970). Twenty-seven mice were infected with *P. berghei* (10^7) PRBCs by ip injection on the first day (D_0). Seventy-two (72) hours later (D_3), the mice were randomLy divided into nine groups of three mice each. Seven groups of the mice were treated orally with 100 $mgkg^{-1}$bwt of the various VLC subfractions respectively. The negative control group was treated with 5% Tween 20 while the positive control groups were treated with Artemether/Lumefantrin (20/120 mg/Kg). The samples and the standard drugs were each treated once daily for four days. Giemsa-stained thin smears were prepared from tail blood samples collected on the 5^{th} day (D_8) to monitor parasitaemia level. The survival time of the

animals in each treatment group was determined over a period of 10 days ($D_0 - D_{13}$) (Basir *et al.*, 2012; Ryley and Peters, 1970).

3.2.7 TOXICITY STUDIES ON *B. PUBESCENS* ETHYL ACETATE FRACTION

3.2.7.1 Acute toxicity study

Evaluation of the median lethal dose (LD_{50}) and chronic toxicity of the EtOAc fraction of *B. pubescens* were carried out in the Department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Okofia, Nnewi Campus. This was determined using the method of Lorke (1983). In this study, a total of 13 mice were used. They received the extract via oral route and it was carried out in two phases.

- **Phase I:** Nine (9) rats were used and they were grouped into three groups of three mice each. Group 1 received 10 mg/Kg; Group 2 received 100 mg/Kg; and Group 3 received 1000 mg/Kg. The animals were observed over a period of 24 h for mortality and there was none. The animals without obvious toxixity signs were progressed to the second phase. In this phase, 4 mice were used and they were grouped into four groups of one animal each per group.
- **Phase II:** Group 1 received 1200 mg/Kg, Group 2 received 1600 mg/Kg**,** Group 3 received 2900 mg/Kg, and Group 4 received 5000 mg/Kg. The animals were monitored over a period of another 24hrs for mortality. $LD_{50} = \sqrt{a \times b}$

Where a= maximum dose with 0% mortality; b= minimum dosed with 100% mortality

The LD_{50} of Ethyl acetate Fraction of *B. pubescens* was above 5000 mg/Kg.

Table 7: Acute toxicity study

PHASE	DOSE	DEATH	OBSERVATION
1	10 mg/Kg	0/3	----------
	100 mg/Kg	0/3	----------
	1000 mg/Kg	0/3	……..
2	1200 mg/Kg	0/1	The animal was calm
	1600 mg/Kg	0/1	The animal was calm
	2900 mg/Kg	0/1	The animal was calm
	5000 mg/Kg	0/1	The animal was calm

3.2.7.2 Chronic toxicity studies

3.2.7.2.1 Experimental design and sample collection

Sixty male Wistar Rats weighing between 150-250 g were used for this study. The animals were acclimatized for a period of two weeks, and then randomly assigned into 4 groups of 15 animals each. Group 1 served as control (animals received only distilled water and laboratory chow); Group 2 received 250mg/Kg of the EtOAc fraction of *B. pubescens*; Group 3 received 500mg/Kg of the EtOAc fraction of *B. pubescens*; and Group 4 received 1000mg/Kg of the EtOAc fraction of *B. pubescens.*

The administration of the extract was between 8 to10 am daily for a period of 16 weeks. The animals were sacrificed after the 16^{th} weekand their blood collected for liver and kidney function tests, and also for haematological tests.

3.2.7.2.2 Sample collection

The livers and kidneys of the test animals were harvested and stored in 10% formalin as a preservative in a container before taking to histopathologist for histopathological investigations.

3.2.7.3 Estimation of haematological indcies

3.2.7.3.1 Total white blood count

The total white blood cells (WBCs) were enumerated by the method of Robert (1993). Blood was drawn exactly to the 0.5 mark in a white blood cell diluting pipette. Immediately, diluting fluid (glacial acetic acid – 2 cc, gentian violet – 0.025 g, distilled water made up to l00mL) was also drawn to the 11 mark. The content of the pipette was mixed for 3-5 min, and a drop was placed at the junction of the cover glass and the Neubauer counting chamber. The white cells

where counted in the four large corner squares containing sixteen smaller secondary squares, each with an area of 0.04 mm^2.

$$\text{WBC per cu mm} = \frac{\text{Number of WBC X dilution (20)}}{\text{Volume (0.4)}}$$

3.2.7.3.2 Estimation of hemoglobin

Hemoglobin was determined by the method of Robert (1993). Five milliliters of buffered cyanide/ferricyanide reagent was pipetted into clean and dry test tubes; 0.02 mL of blood was added to the same tubes. The pipette was thoroughly flushed with reagent. The solution was mixed well and incubated at 30°C. Absorbance A of sample was read against distilled water after 3 min at 546 nm.

The concentration 'C' of hemoglobin was calculated as follows;

C = 36.77 × A (g/100 mL)

3.2.7.3 .3 Estimation of packed cell volume

Pack cell volume (PCV) was determined by the method established by Robert (1993). The capillary tube was filled up to two-third (2/3) to three-quarters (3/4) full with well-mixed oxalated venous blood. One end of the tube was sealed with plasticine, and the filled tube was placed in the micro-hematocrit centrifuge, with the plugged end away from the center of the centrifuge. This was then centrifuged at a preset speed of 10000 to 12000 rpm for 5 min. Lastly, the spine tube was placed in the micro-hematocrit reader and the value taken.

3.2.7.3.4 Estimation of red blood cells

Red blood cell was determined by the method established by Robert (1993). Cardiac puncture blood was drawn from the rats and put in a RBC pipette up to 0.5 mark. RBC diluting fluid was

taken up to 101 mark in single mark pipette or RBC pipette and rotated equally to mix the solution well by swirling. The haemocytometer was placed on the flat surface of the work bench and the cover slip was placed on the counting chamber and a small drop of diluted blood, hanging from the pipette, was allowed to sweep into the counting chamber by capillary action. Precaution was taken to make sure that there was no air bubble and there was no overfilling beyond the ruled area. The counting chamber was left on the bench for 3 min to allow the cells to settle. The cells were observed by placing the counting chamber on the mechanical stage of the microscope. Focused on the center room of the chamber, the cells were counted from upper left corner of the room.

Data analysis:

$$\frac{\text{No. of cells x Dilution factor x Depth factor x Total ruled area}}{\text{Area count}}$$

Where;

Dilution factor = 200, Depth factor = 10

Total ruled area = 25, Area count = 5

3.2.7.4 Kidney function tests

3.2.7.4.1 Creatinine estimation

This test was carried out according to standard method (Bancroft and Gamble 2002; Thavarajah *et al.,* 2012; Alturkistani *et al.,* 2016). Creatinine present in the serum or plasma reacts with alkaline picrate resulting in the formation of a yellow-red color. The intensity of the colour determines the concentration of creatinine in the sample. The Absorbance of the yellow-red colour produced was measured using a spectrophotometer measured at 520 nm wavelength.

Protein interference is eliminated using sodium laurylsulphate. A second absorbance reading after acidifying with 30% acetic acid corrects for non-specific chromogens in the samples.

3.2.7.4.2 Urea estimation

This test was carried out according to standard method (Bancroft and Gamble, 2002; Thavarajah *et al.,* 2012; Alturkistani *et al.,* 2016). Urea in serum is hydrolyzed to ammonia in the presence of the urease. The ammonia reacts with phenol and hypochlorite in alkaline medium to form indophenol. The intensity of the colour indicates the concentration of urea in the sample. Nitroprusside is used to catalyze the reaction. This indophenol is then measured photo metrically at 530-570nm wavelength.

3.2.7.5 Liver function tests

3.2.7.5.1 Alkaline Phosphatase (ALP) estimation

This test was carried out according to the method described by Kind and Jegathessan (1953). Alkaline phosphatase in alkaline medium hydrolyses phenyl phosphatase in 15min at 37°C and pH of 10 to release phenol which in the presence of potassium ferricyanide reacts with 4-aminophenazone to give a red-pink colour which is measured spectrophotometrically at 510nm wavelength. The intensity of the colour indicates ALP activity in the sample.

3.2.7.5.2 Aspartate Amino Transferase (AST) estimation

This test was carried out according to the method described by Reitman and Frankel (1957). The substrates in the reaction are alpha ketoglutaric acid and L- Aspartate. The products formed by enzyme action are glutamate and oxaloacetate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition

of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using spectrophotometer.

3.2.7.5.3 Alanine Amino Transferase (ALT) Estimation

This test was carried out according to the method described by Reitman and Frankel (1957). The substrates in the reaction are alpha ketoglutaric acid and L-aspartate. The products formed by enzyme action are glutamate and pyruvate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using spectrophotometer.

3.2.7.6 Tissue Processing for Photomicrography

Tissue sections were produced by normal histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting, and staining. The micrographs of relevant stained sections were subsequently taken with a photomicroscope.

1. Fixation: After weighing the organs, a small part was cut from each of them and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction.

Fixation is a very important step in tissue processing as it does not only protect the tissue from autolysis and putrefaction but also hardens the tissue to withstand other chemicals applied in subsequent treatments and for easy handling.

2. Dehydration: After fixation, the tissues were transferred and dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95% and 100% or absolute alcohol once for 2 h each but twice in absolute alcohol).

The tissues were placed in ascending grades of alcohol to prevent distortion and distortion to the cell structure would happen if directly placed in absolute alcohol. However, sufficient time was allowed in absolute alcohol to enable complete dehydration (Kim *et al.,* 2017; Dapson *et al.,* 2010).

3. Clearing: The tissues were cleared twice in xylene for 1 to 2 h each time. This is to avoid over exposure in the clearing agent, which will make them brittle. Xylene was used as the clearing agent as it does not only remove alcohol but is equally miscible with paraffin used in embedding.

3. Impregnation: The tissues were placed in molten paraffin wax at a constant temperature of 56^{o}C (3^{o}C above the melting point of paraffin wax used) in an oven and were passed through two changes of paraffin wax in the oven, 4 h each. This was done to replace the clearing agent or antemedium with molten paraffin wax and can also be referred to as *infiltration.* The tissues were subsequently removed from the oven and embedded in paraffin wax (Albert *et al* 2002; Wisse *et al* 2010).

4. Embedding: Embedding is a process of burying a tissue in molten paraffin wax. The paraffin becomes a solid firm structure when it is cold, and this forms a support medium for the tissue during microtomy. The tissues were then immersed in molten paraffin wax at a constant temperature of 36^{o}C to 60^{o}C in an oven of paraffin bath changing it twice for 2-4 h each time. They were left to cool and solidify in metallic embedding moulds. The tissue blocks obtained were casted on to the wooden blocks for sectioning (Alturkistani *et al.,* 2016).

5. Sectioning: This was done using a Rotatory Microtome. The tissue blocks were mounted on wooden blocks. With the microtome knife and blocks positioned accurately, sections were made at 5 microns each. The ribbons of sections were floated in warm water bath (37^{o}C) to straighten

them. The best ribbons were picked with forceps and placed on albuminised slides. The slides were labeled using diamond pencils and transferred to a slide rack. They were then placed in an oven to keep the specimens warm before staining.

6. Staining: The tissues were stained using Ehrlich's Haematoxylin and Eosin stains. The staining procedure is as follows: The slides treated with paraffin wax were cleared in xylene for 3 min, and were rehydrated in descending grades of alcohol; i.e. absolute alcohol for 2 min to remove xylene, 90% alcohol for 2 min, 70% alcohol for 2 min, 30% alcohol for 2 min, and then rinsed in water for 1 min. The tissues were stained by immersing them in aqueous solution of haematoxylin for 30 min, and then rinsed in water to remove excessive stains. The tissues were subsequently differentiated in 1% acid alcohol for 1 min. This process called *Bluing* gave the tissues their characteristic blue background. The tissues were then stained in aqueous eosin for 10 min. The tissues were now immersed in ascending grades of alcohol as follows; 50%, 70%, 90% and absolute alcohol for I min each and then cleared in xylene for 1 min. Staining gives contrasting colours to different elements of the cells or tissue thus making them conspicuous and easy to study.

7. Mounting: The slides were removed from the rack through their edges with the aid of forceps and placed on the filter papers. Blotting was done in one direction on the filter papers using the index finger and few drops of xylene were placed on the slides to make them wet. A drop of Dibuty Phthalate Xylene (DPX) mountant was placed on the slide which was laid in the middle to minimize the likelihood of trapping air bubbles. The slides were quickly inverted over cover slip and then brought down horizontally until the mountant made contact.

3.2.8 STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Sciences (SPSS Version 23). The results were expressed as mean ± SEM. Data for hematological parameters, kidney function test (creatinine and urea), liver function tests (ALT, AST and ALP) were analyzed using One-way ANOVA, followed by post hoc Least Significant Difference (LSD). While body weight were analyzed using Student dependent T-test. Values were considered significant at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1 ACUTE TOXICITY TEST ON THE DIFFERENT PLANTS

The results of the acute toxicity test on the different plants are presented in Table 8. The methanol extract caused no death after the second stage of acute toxicity test carried out using Lorke method (Lorke, 1983). The LD_{50} was therefore estimated to be greater than 5000 mg/Kg and considered safe for acute administration of all the plants tested.

Table 8: Result of acute toxicity test on the different plants

Phase	Concentration (Mg/Kg)	Observation
1	10	0/3 - no death
	100	0/3 - no death
	1000	0/3 - no death
2	1600	0/1 - no death
	2900	0/1 - no death
	5000	0/1 - no death
Control	DMSO:Water ratio (1:1) 1 mL/kg	0/1 - no death

4.2 PRELIMINARY ANTIMALARIAL ACTIVITY OF THE DIFFERENT PLANTS

The result of the preliminary antimalarial activity of crude extracts of the selected plants, showing their percentage cures is shown in Figure 12. Of all the plants tested, *B. pubescens* showed best antimalarial activity and was chosen for further studies.

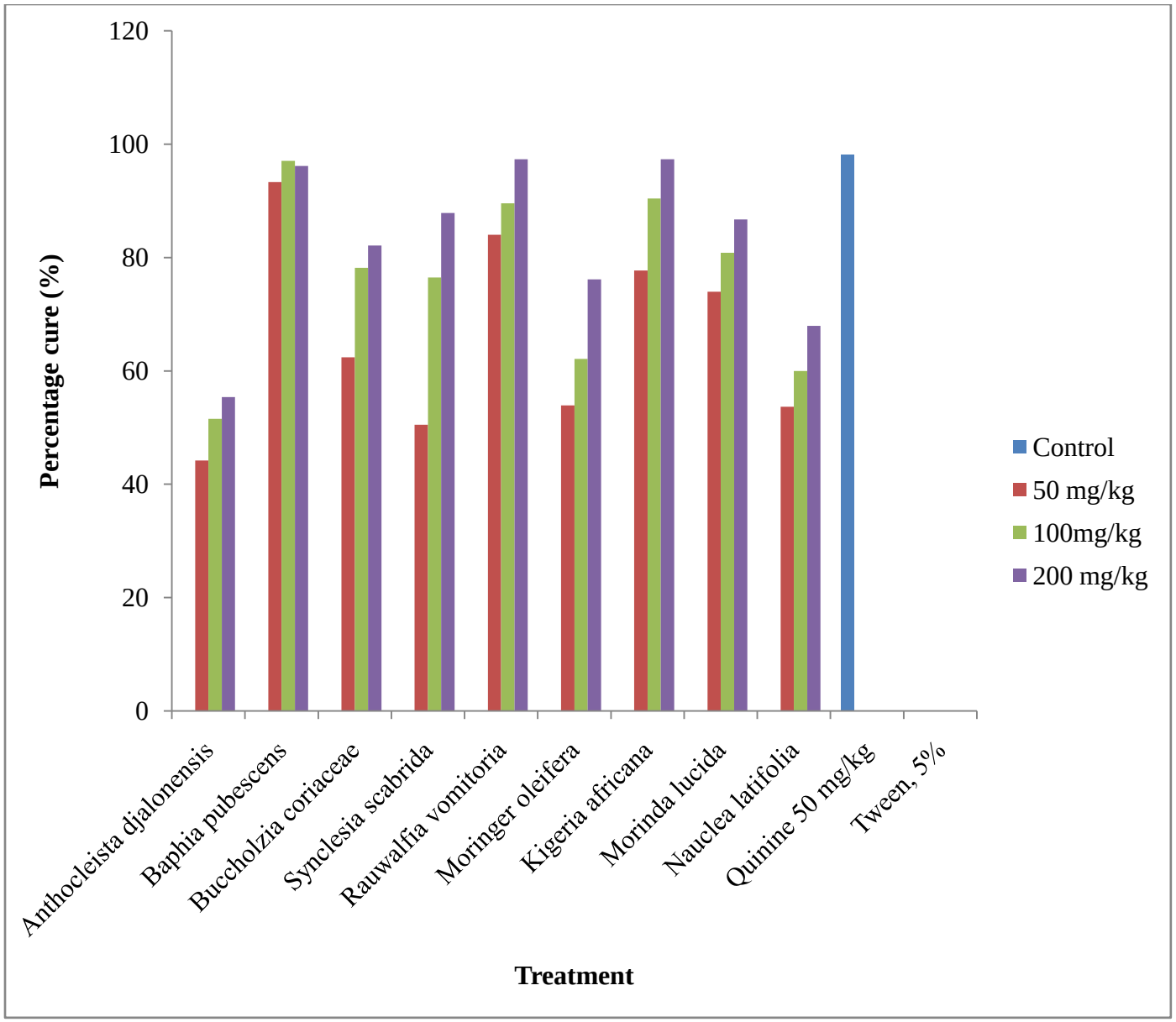


Figure 12: Preliminary antimalarial activity of crude extracts of the selected plants, showing their percentage cures

4.3 PHARMACOGNOSTIC STUDIES ON *B. PUBESCENS*

Data on the leaf measurements of *B. pubescens* including the stomatal number, stomatal index, vein-islet number, veinlet termination number, and palisade ratio are presented in Tables 10. Table 11 shows the extractive values of *B. pubescens.*

Results of the determination of the percentage phytochemical content and results of the phytochemical analysis of *B. pubescens* are presented in Tables 12 and 13 respectively.

Results of microscopic examination of *B. pubescens* leaves are shown in Figures 13-17. Also, photomicrographs of the general anatomy of *B. pubescens* leaves are shown in Figures 19-24.

4.3.1 Leaf Measurements

1. Stomatal number - this is the average number of stomata per square millimetre of epidermis.

2. **Vein-islet number** - the term vein-islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of veins-islets mm^{-2} calculated from four contiguous square millimetres in the central part of the lamina midway between the mid-rib and the margin is called the vein-islet number.

3. **Veinlet termination number** - this is the number of veinlet terminations per mm^{-2} of leaf surface.

4. **Palisade ratio** - This is the average number of palisade cells beneath each upper epidermis.

Calculations of *B. pubescens* stomata number, vein islet number, veinlet termination number, and palisade ratio are presented in Table 9.

Table 9: Calculation of *B. pubescens* stomata number, vein islet number, veinlet termination number, and palisade ratio

Specimen	Stomata Number	Vein Islet Number	Veinlet Termination Number	Palisade Ratio
1	35	7/3 = 2.33	5/3 = 1.67	35/4 = 8.75
2	32	9/3 =3.00	6/3 = 2.00	38/4 =9.50
3	35	8/3 = 2.67	6/3 = 2.00	36/4 = 9.00
Average	34	2.66	1.89	9.08

5. **Stomatal index** - this is the percentage of the ultimate divisions of the epidermis of a leaf which has been converted into stomata -

$$\mathrm{I} = \frac{\mathrm{S}}{E+S} X \frac{100}{1}$$

Where S = number of stomata per unit area; E = number of ordinary epidermal cells in the same unit area

Therefore;

Sample 1

S = 36

E = 176

$$\mathrm{I} = \frac{36}{176+36} X \frac{100}{1} = 16.98$$

Sample 2

S = 32

E = 169

$$\mathrm{I} = \frac{32}{169+32} X \frac{100}{1} = 15.92$$

Sample 3

S = 35

E = 180

$$\mathrm{I} = \frac{35}{180+35} X \frac{100}{1} = 16.28$$

Average Stomatal Index $\mathrm{I}^{a} = \frac{16.98+15.92+16.28}{3} = 16.39$

Table 10: *B. pubescens* extractive values

Parameter	**Value (%)**
Alcohol extractive value	32.65
Water extractive value	5.45
Moisture content	8.5
Total ash	3.35
Water soluble ash	0.55
Acid insoluble ash	1.52

Table 11: *B. pubescens* percentage phytochemical content

Parameters	**% phytochemical content**
Glycoside	31.86
Flavanoids	21.89
Saponins	10.11
Tanins	11.11
Phenols	28.89

4.3.2 Microscopic Examination of *B. pubescens*

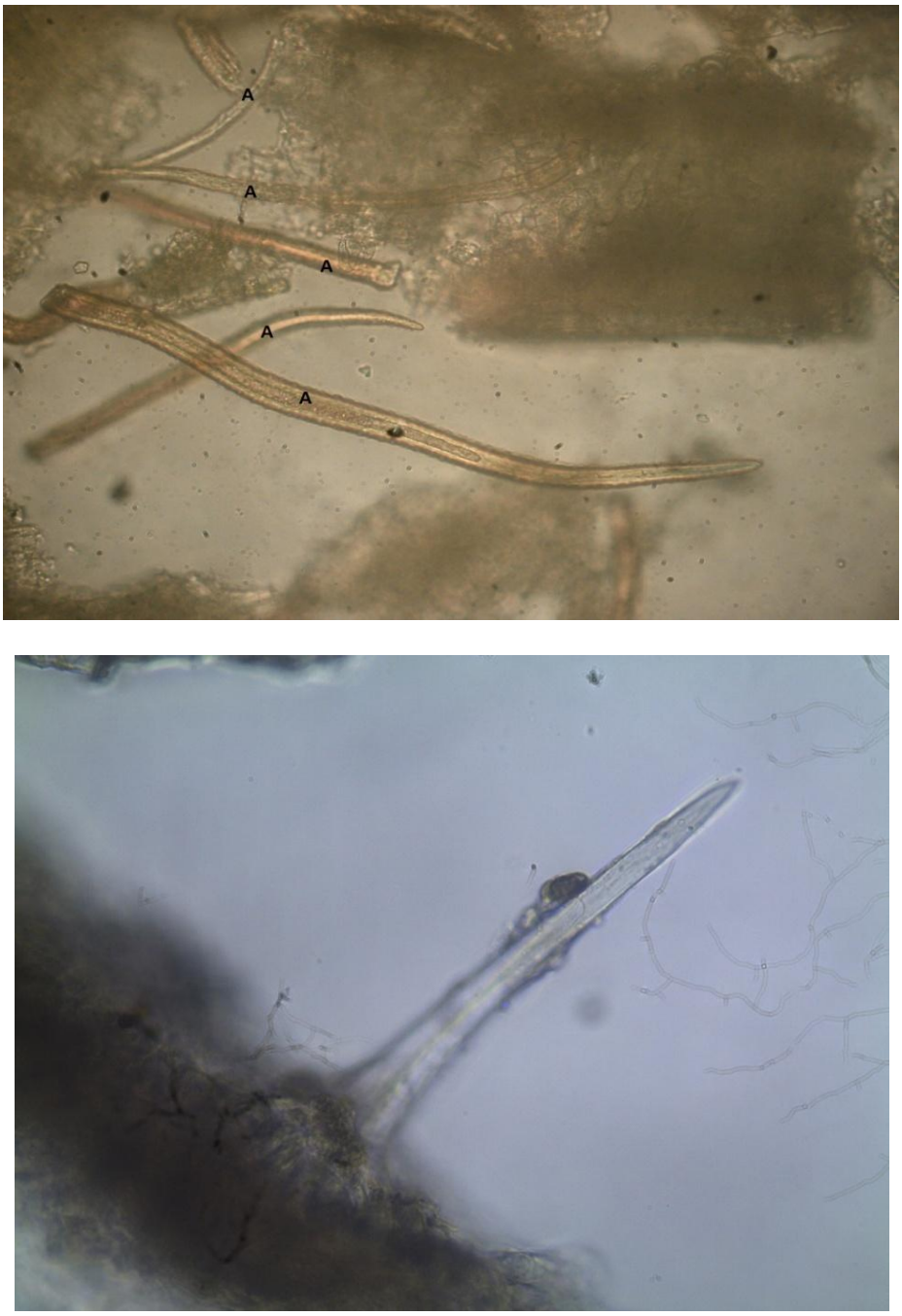


Figure 13: Photomicrograph of *B. pubescens* leaf powder showing unicellular trichomes X400.

KEY

A = Unicellular trichome

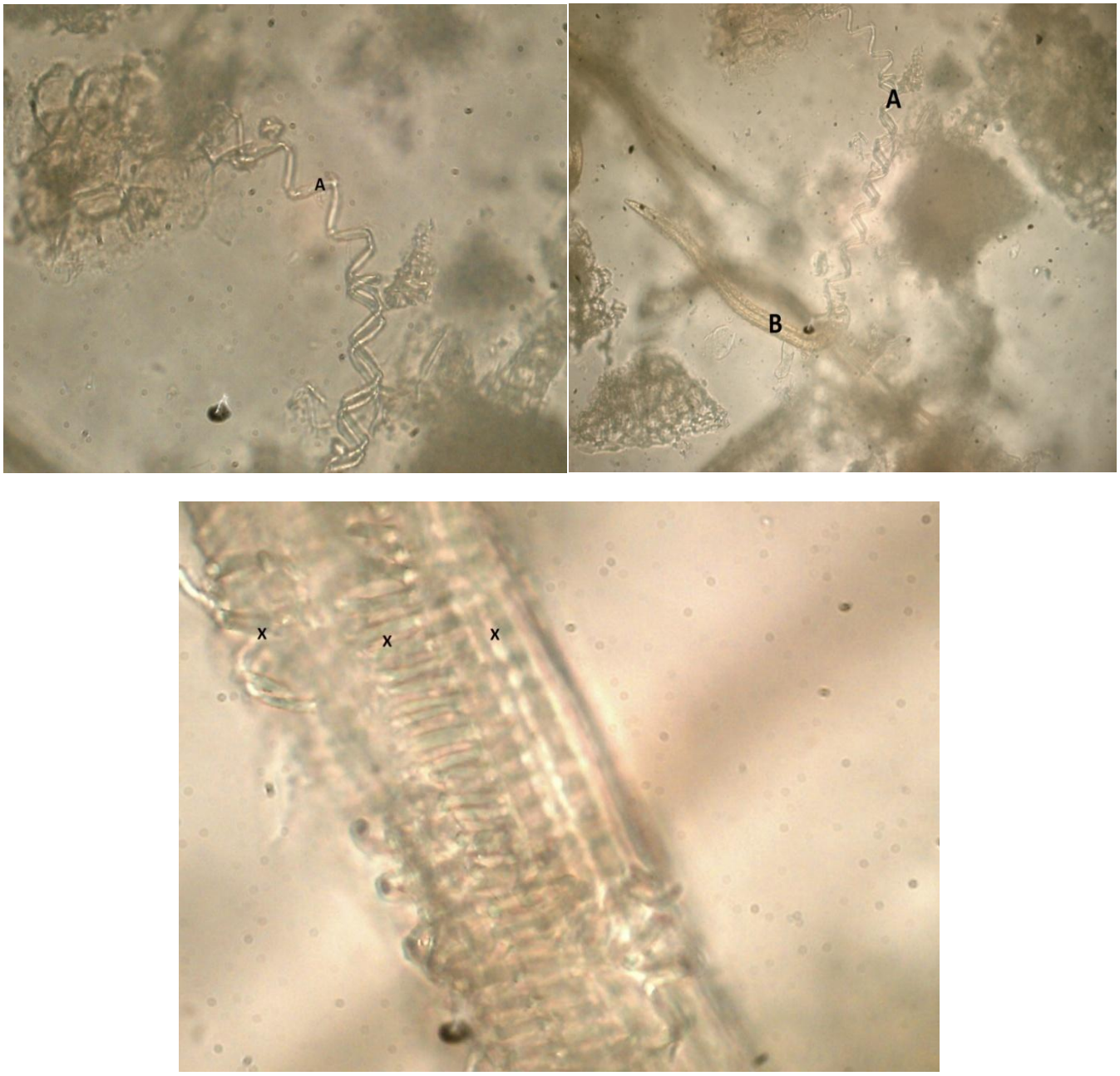


Figure 14: Photomicrograph of *B. pubescens* leaf powder showing spiral xylem vessels and unicellular trichome X400.

KEY

A and X = Spiral xylem vessel

B = Unicellular trichome

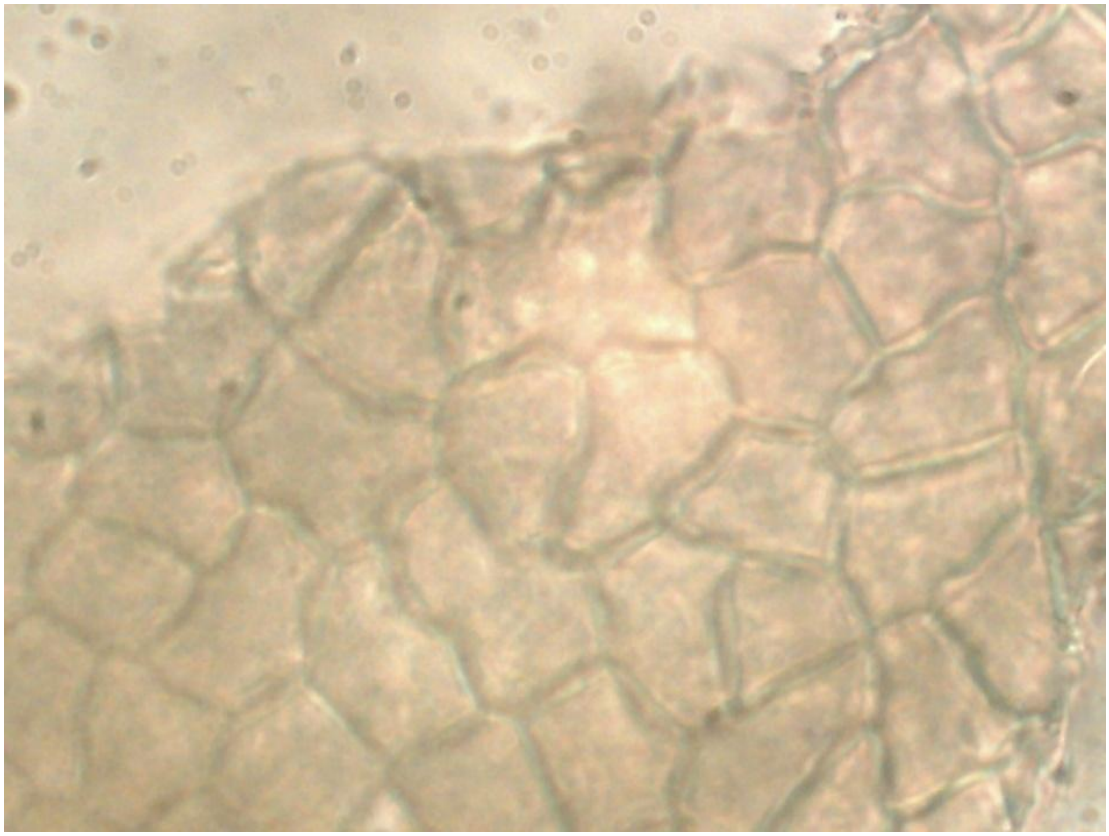


Figure 15: Photomicrograph of the upper epidermis of *B. pubescens* powdered leaf showing straight walled epidermal cells X400.

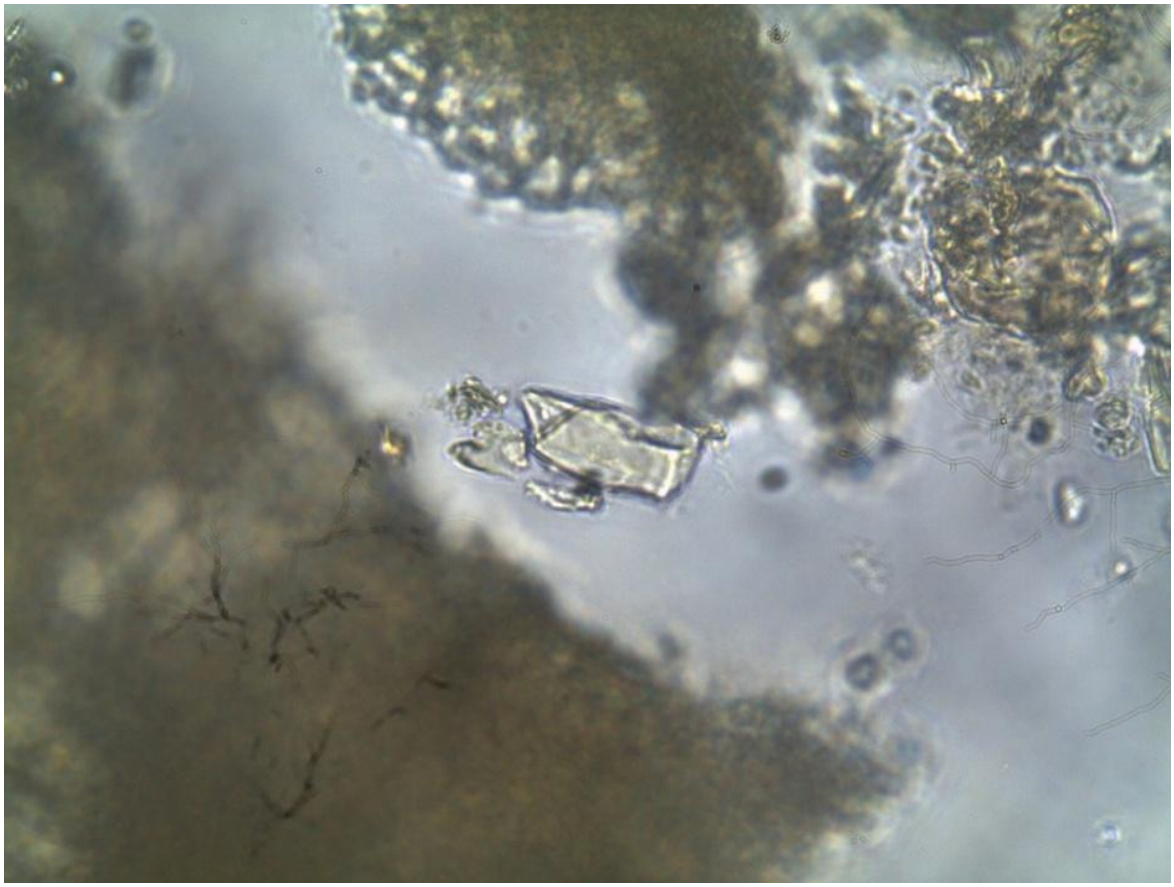


Figure 16: Prism shape calcium oxalate X400.

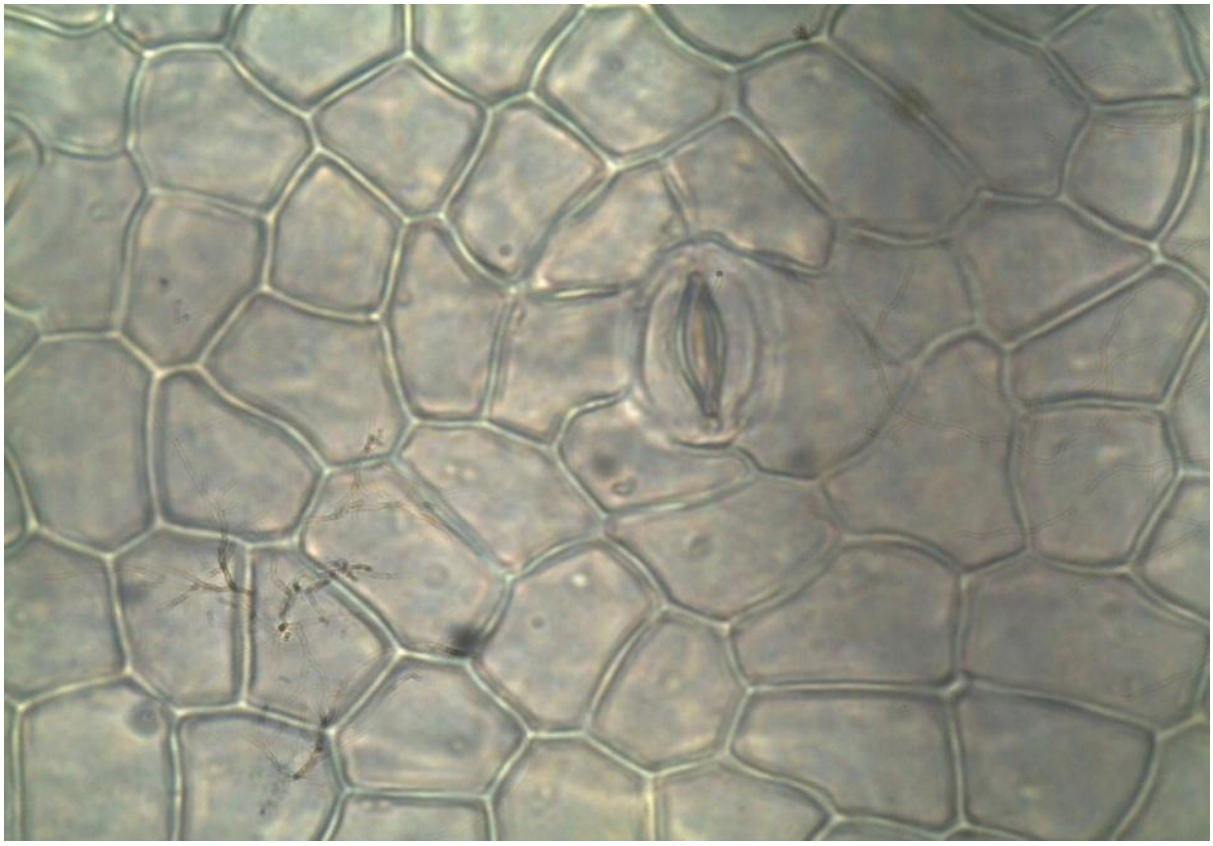


Figure 17: Anomocytic stoma X400.

4.4.3 General Anatomy of *B. pubescens*

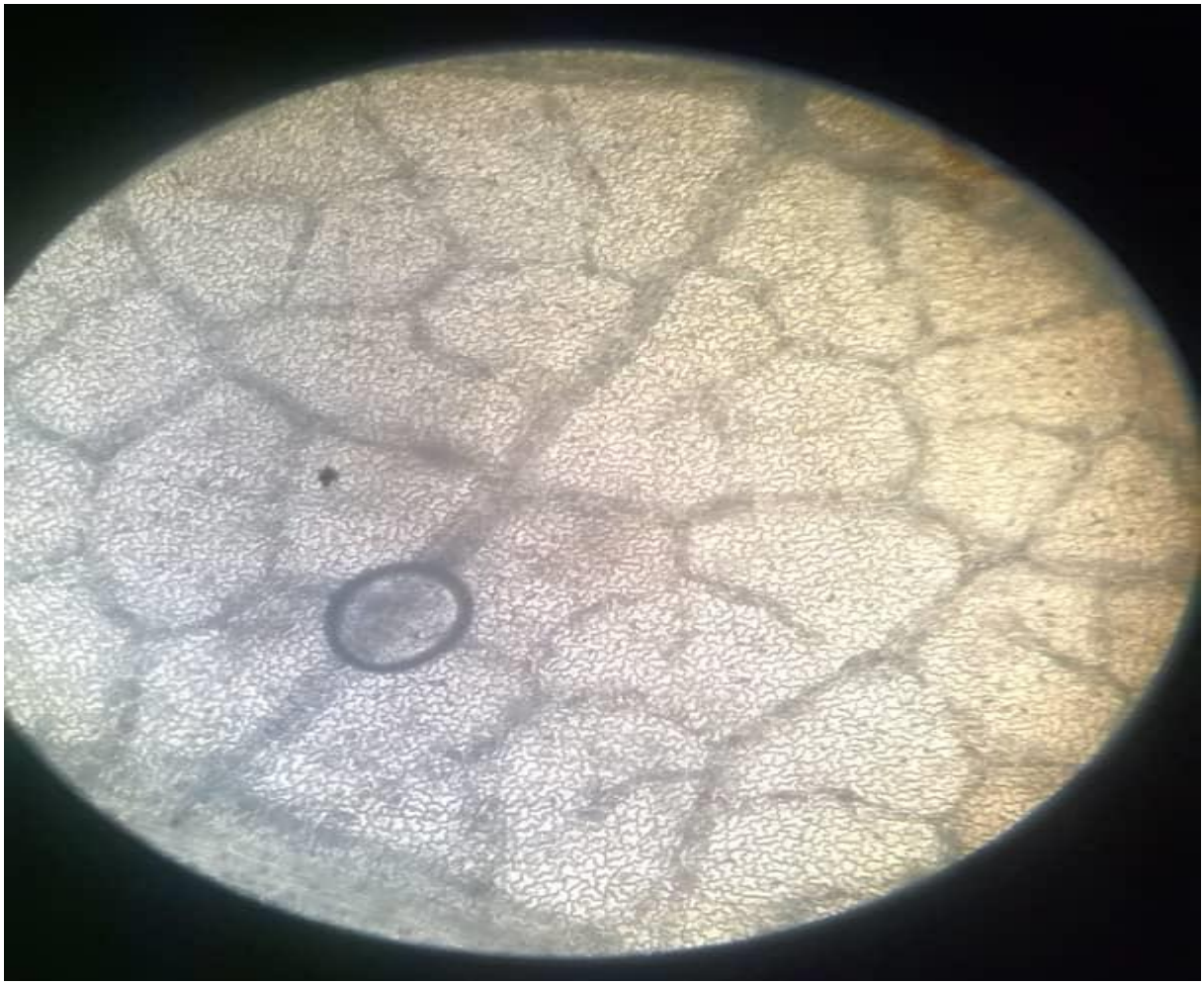


Figure 18: Anatomy of *B. pubescens,* showing lower epidermis in surface view

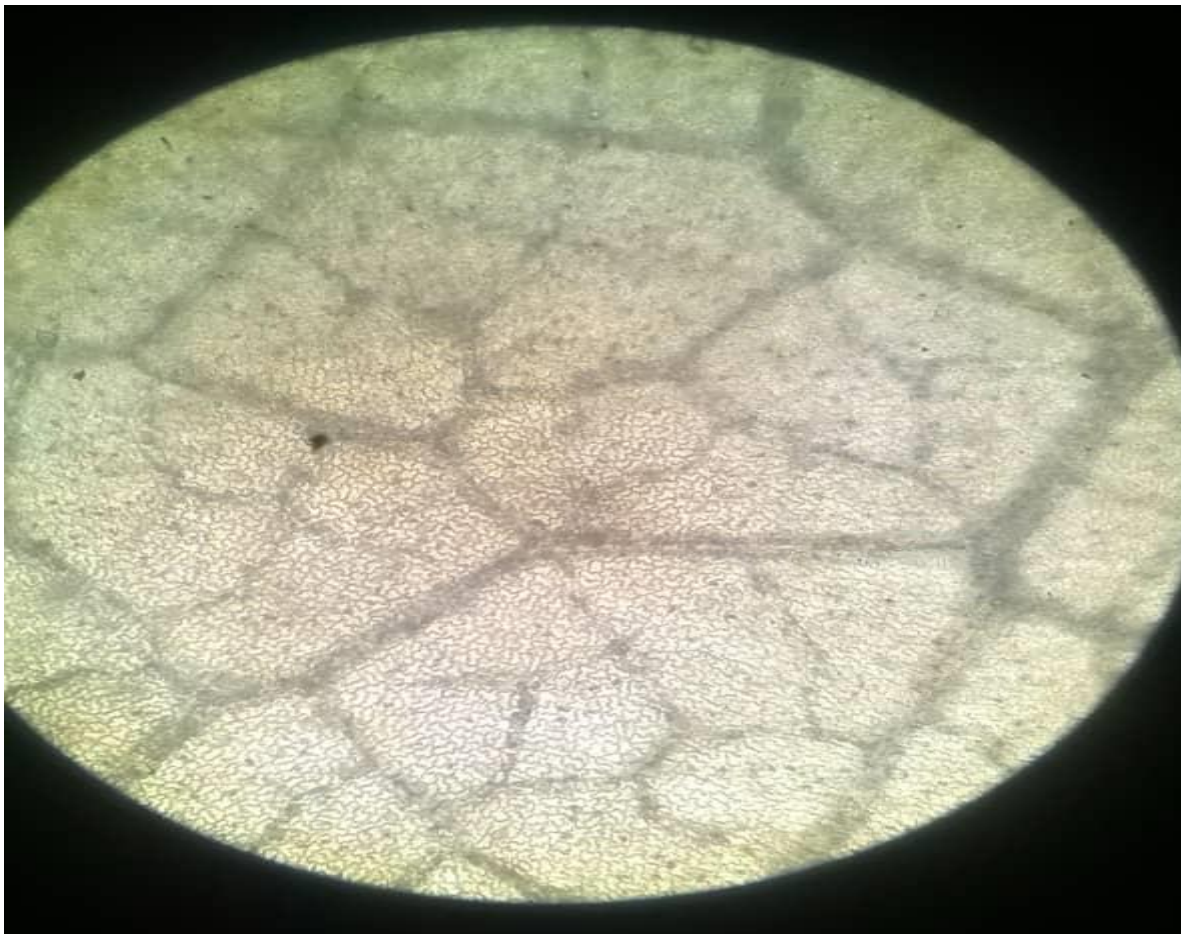


Figure 19: Anatomy of *B. pubescens,* showing upper epidermis in surface view, with prisms of calcium oxalate

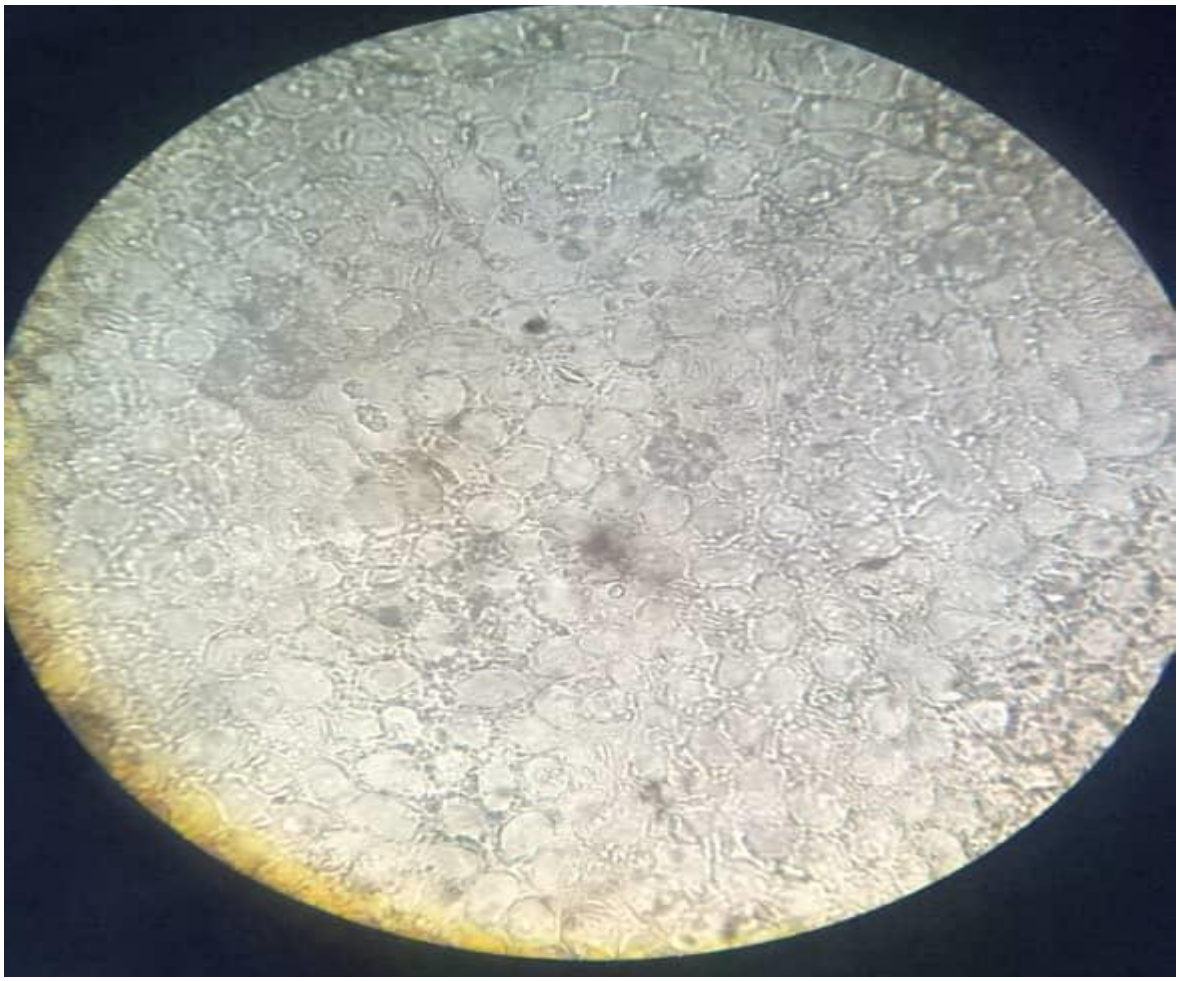


Figure 20: Anatomy of *B. pubescens,* showing part of the lamina in sectional view showing the lower epidermis, palisade, spongy mesophyll with cluster crystals of calcium oxalate, part of a fibre and crushed sieve tissue.

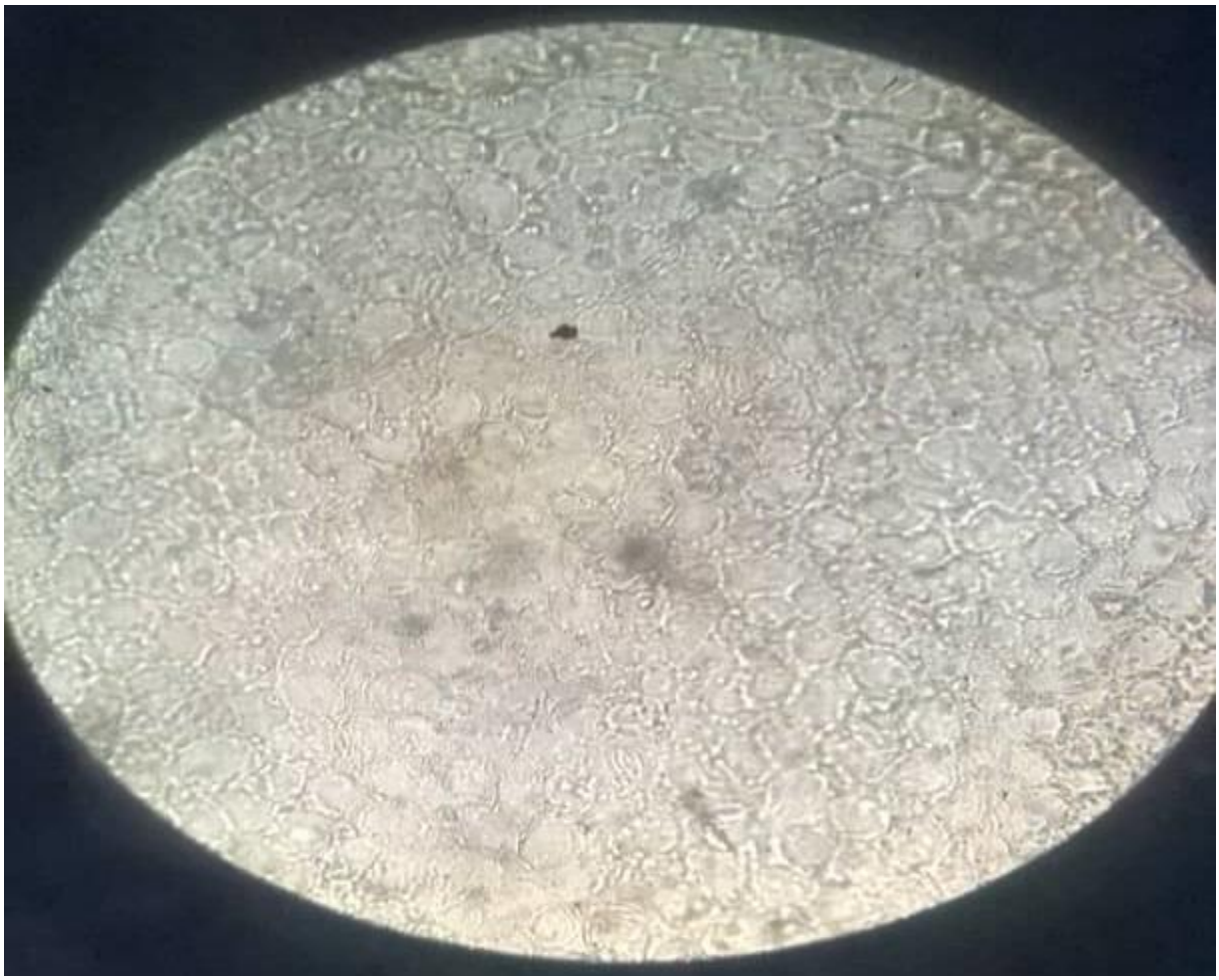


Figure 21: Anatomy of *B. pubescens,* showing the polygonal, straight-walled parenchyma of the perigone

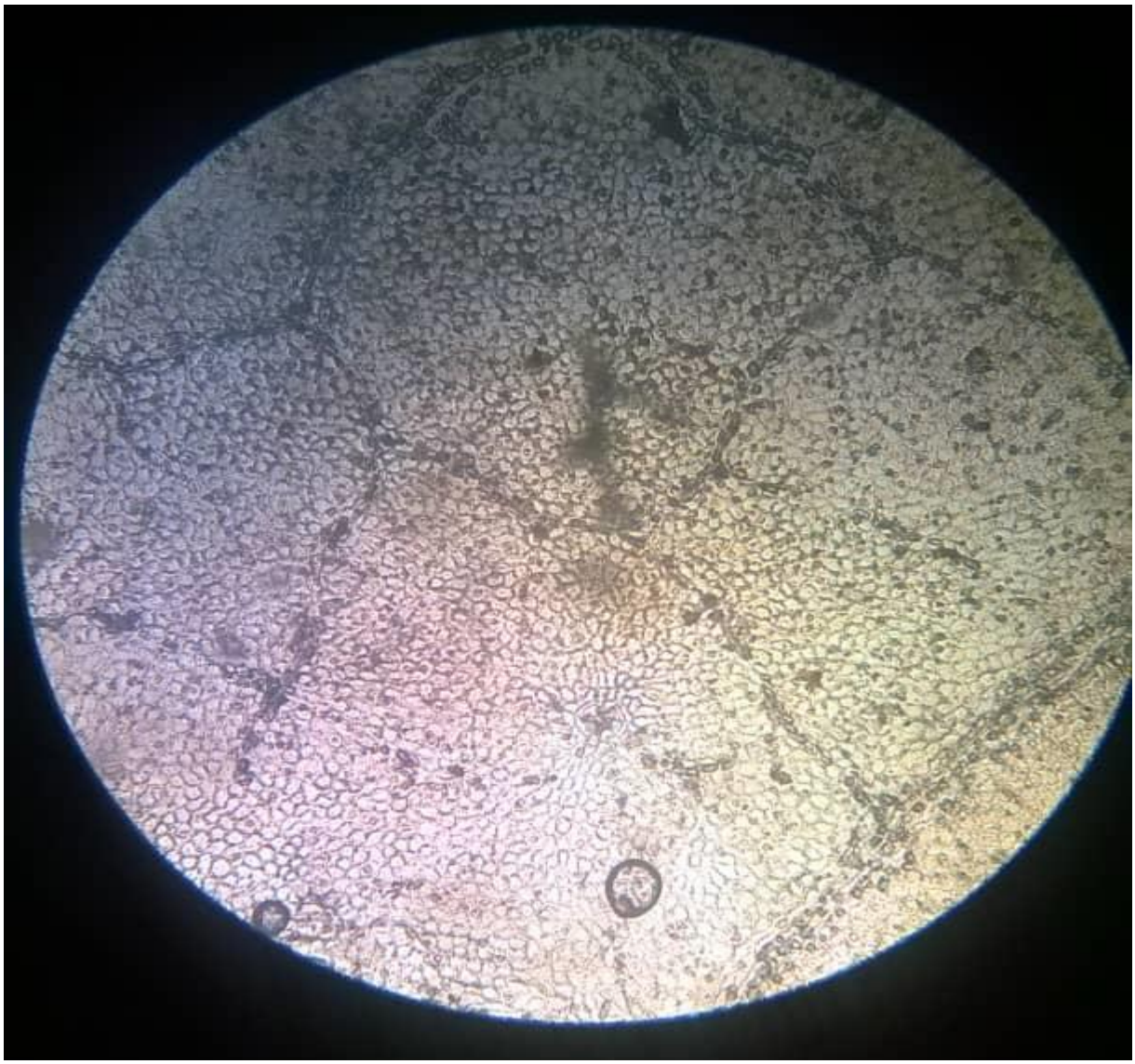


Figure 22: Anatomy of *B. pubescens,* showing pollen grains

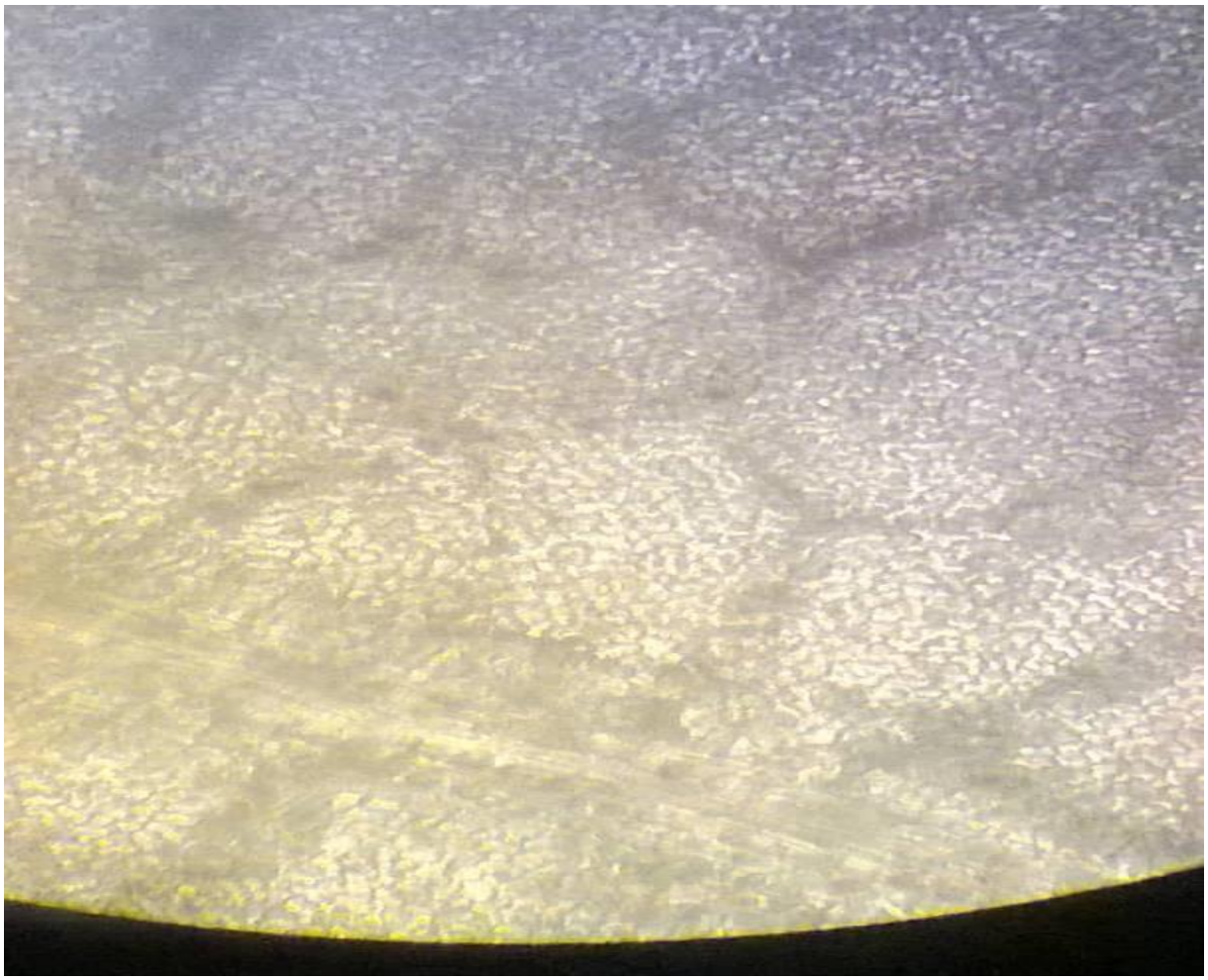


Figure 23: Anatomy of *B. pubescens,* showing cells of the spongy mesophyll in surface view containing dense brown pigment, with part of the epidermis lying underneath

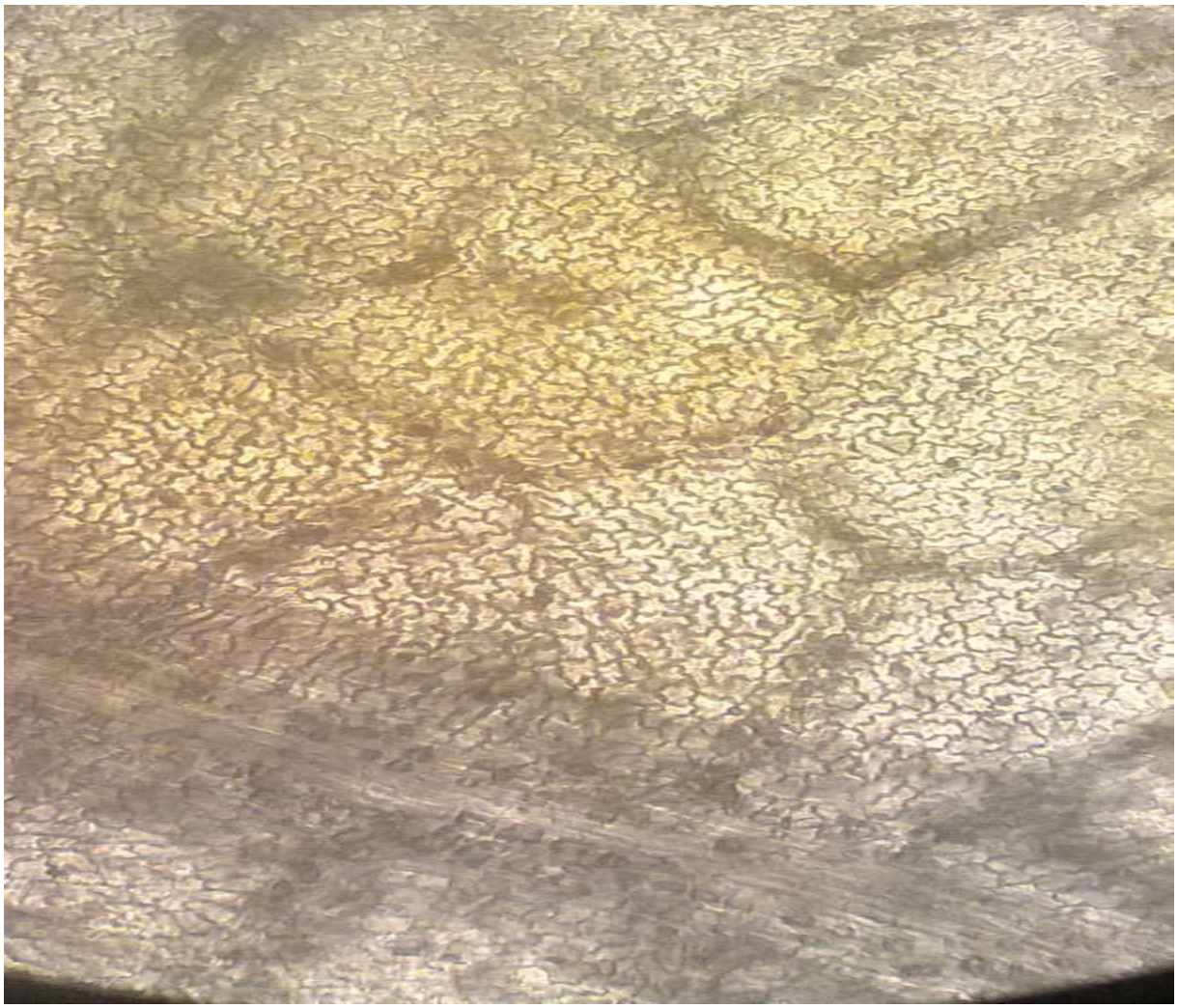


Figure 24: Anatomy of *B. pubescens* showing cluster crystals of calcium oxalate

4.4 ANTIMALARIAL ACTIVITY OF THE DIFFERENT FRACTIONS OF *B. PUBESCENS*

The result of the antimalarial screening of the different fractions of *B. pubescens* crude extract is shown in Figure 25. From their percentage cures, it can be observed that the ethyl acetate activity exhibited best activity (95.36%) which is comparable to that recorded for the positive control quinine (98.11%).

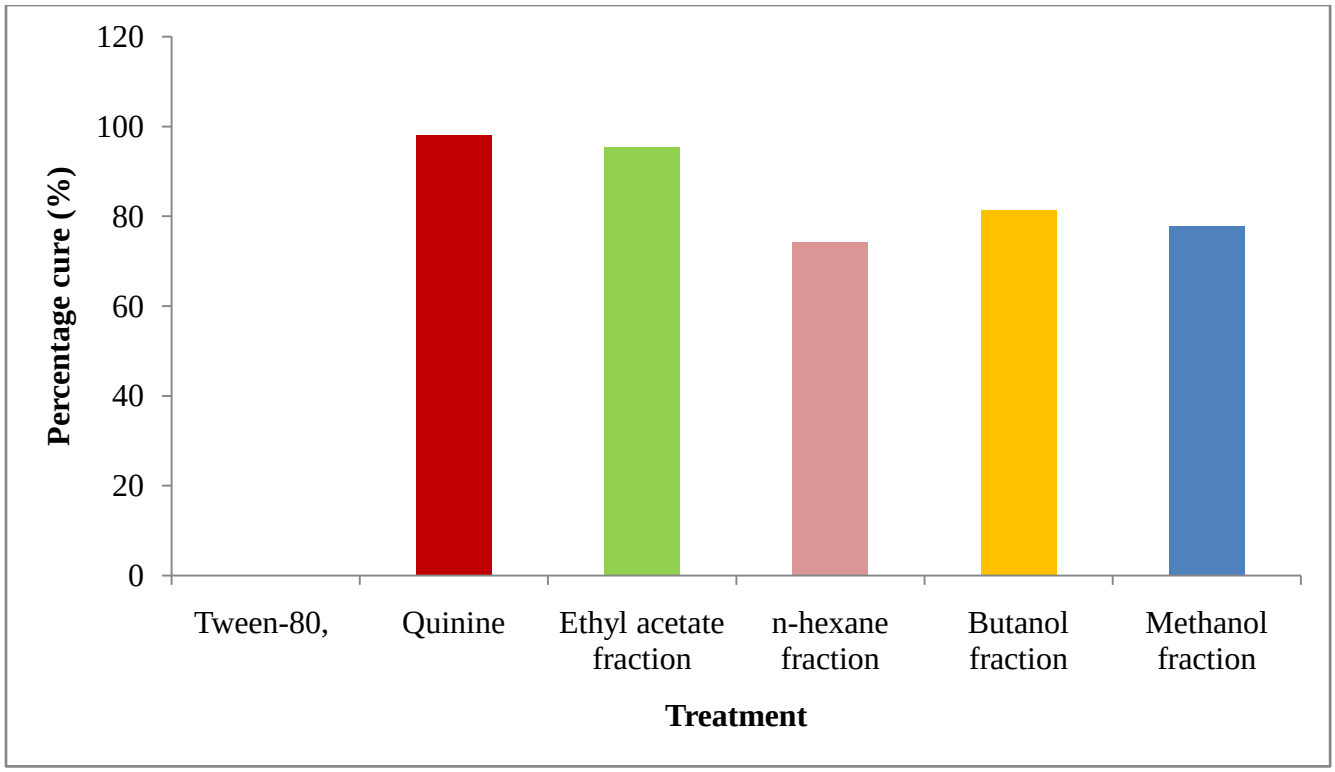


Figure 25: Antimalarial activity of the different fractions of *B. pubescens* crude extract, showing their percentage cures

4.5 CHROMATOGRAPHIC ANALYSIS - VLC RESULTS

Information on the solvents mixtures and weight of fractions obtained from the VLC of the ethyl acetate fraction of *B. pubescens* is presented in Table 12. Also, information on the various batches of factions obtained from Sephadex separation is presented in Table 13.

Table 12: Ratios of solvents mixtures and weight of fractions obtained

S/N	*n*-Hex: EtOAc	Volume (mL)	Weight (mg)
1	10:0	500:0	46
2	9:1	450:50	593
3	8:2	400:100	2000
4	7:3	350: 150	952
5	6:4	300;200	543
6	5:5	250:250	400
7	4:6	200:300	323
8	3:7	150:350	264
9	2:8	100:400	227
10	1:9	50:450	66
11	0:10	0:500	163
	DCM:MeOH	**Volume (mL)**	**Weight (mg)**
12	10:0	500:0	74
13	8:2	400:100	252
14	5:5	250:250	736
15	2:8	100: 400	97

Table 13: Weight of various batches of factions obtained from Sephadex separation

Sample name	Weight of empty tube with cover (g)	Weight of samples and empty tube with cover (g)	Weight of samples (g)
FRED 1	1.4916	1.5800	0.0884
FRED 2	1.5257	1.5749	0.0492
FRED 3	1.5684	1.6277	0.0593
FRED 4	1.6209	1.7878	0.1669
FRED 5	1.6437	1.7562	0.1125
FRED 6	1.5017	1.5913	0.0896
FRED7	1.5782	1.6784	0.1002
FRED 8	1.4877	1.5403	0.0526
FRED 9	1.6574	1.7039	0.0465
FRED 10	1.4850	1.5624	0.0774
FRED 11	1.5719	1.6000	0.0281
FRED 12	1.5353	1.5793	0.0440
FRED 13	1.5116	1.5462	0.0346
FRED 14	11.6217	1.6812	0.0595
FRED 15	1.5625	1.6256	0.0631
FRED 16	1.5704	1.5908	0.0204
FRED 17	1.5501	1.5902	0.0401
FRED 18	1.4892	1.5885	0.0993
FRED 19	1.4917	1.5450	0.0533

4.6 ANTIMALARIAL ACTIVITY OF THE VLC SUBFRACTIONS OF ETHYL ACETATE FRACTION OF *B. PUBESCENS*

Results of antimalarial assay of the VLC subfractions of *B. pubescens* ethyl acetate fraction are presented in Table 14.

Table 14: Curative test of the of VLC subfractions of ethyl acetate fraction of *B. pubescens*

Sample code	Animal No.	Weight (g)	Parasitaemia (Day 3); Before Treatment	Parasitaemia (Day 8); After Treatment	Fraction Alive after Day 13
D1	1	21	60	-	0/3
	2	20	63	68	
	3	19	68	-	
D2	1	18	70	63	2/3
	2	20	62	38	
	3	21	54	-	
D5	1	22	65	11	2/3
	2	22	59	34	
	3	20	64	43	
D6	1	24	61	-	1/3
	2	18	67	54	
	3	19	61	48	
D7	1	19	59	33	3/3
	2	23	63	28	
	3	22	72	32	
D10	1	19	74	54	2/3
	2	20	66	21	
	3	21	68	18	
D13	1	21	75	38	1/3
	2	22	63	42	
	3	19	78	53	
Artemether/ lumefantrin	1	22	64	9	3/3
	2	18	63	14	
	3	19	72	29	
5% Tween 20	1	23	74	-	0/3
	2	25	68	-	
	3	19	51	-	

4.7 CHRONIC TOXICITY STUDIES OF ETHYL ACETATE FRACTION OF *B. PUBESCENS*

- **Liver enzymes (ALT, AST and ALP)**

Results of the chronic toxicity study on the ethyl acetate fraction of *B. pubescens* are presented in Figures 26, showing effects of the plant on liver enzymes ALT**,** AST and ALP levels.

All data were analyzed using One-way Anova, followed by multiple comparism using Bonferroni, and data were considered significant at $p<0.05$. *$p<0.05$ shows that it was significant, and **$P<0.05$ shows that it was more significant.

Result from Figure 26 showed that for ALT, there was a significant increase in group 2 (92.50±3.22), 3 (119.00±1.95), and 4 (121.00±2.10) when compared to group 1 (41.25±2.92). For AST, there was a significant increase in group 2 (79.50±6.60), 3 (135.00±5.40), and 4 (233.75±11.43) when compared to group 1 (28.00±1.47). For ALP, there was a significant increase in group 2 (95.00±9.12), 3 (151.50±6.30), and 4 (242.75±10.60) when compared to group 1 (54.25±1.79).

- **Kidney Markers (Urea and Creatinine)**

Toxicity of the plant on the kidney was also studied. The effect of the plant on urea and creatinine levels is presented in Figure 27. All data were analyzed using one-way Anova, followed by multiple comparism using Bonferroni, and data were considered significant at $p<0.05$. *$p<0.05$ shows that it was significant, and **$p<0.05$ shows that it was more significant.

Result from Figure 27 showed that there was an increase in urea level when comparing group 1 (44.8±1.73) to group 2 (82.25±5.85), group 3 (132.50±3.22), and group 4 (215.00 ±1.90), but the increase was significant. For creatinine level, there was an increase when comparing group 1

(54.00 ±3.18) to group 2 (100.00±7.35), group 3 (138.75±5.54), and group 4 (228.75±13.28), but the increase was significant.

- **Hematological parameters (RBCs, Hemoglobin, Packed cell volume, WBCs and Platelets**

Effect of the plant on hematological parameters such as the red blood cells, hemoglobin, packed cell volume, white blood cells and platelets were studied and results are presented in Figures 28-31. All data were analyzed using One-way Anova, followed by multiple comparism using Bonferroni, and data were considered significant at $p<0.05$. *$p<0.05$ shows that it was significant, and **$p<0.05$ shows that it was more significant.

From Figure 28, it can be seen that there was an increase in RBC level in group 2 (5.05±0.11), 3 (5.00±0.04), and 4 (6.75±0.10) when compared to group 1 (4.74±0.09), but the increase was only significant in group 4 while that of group 2 and 3 was not significant. For hemoglobin level (Figure 30), there was an increase in group 2 (12.00±0.40), 3 (13.50±0.64), and 4 (15.50±0.65) when compared to group 1 (11.00±0.40), but the increase was only significant in group 3 and 4 while that of group 2 was not significant.

For packed cell volume (Figure 29), there was an increase in group 2 (42.25±1.37), 3 (44.25±1.31), and 4 (47.75±0.47) when compared to group 1 (41.00±0.91), but the increase was only significant in group 4 while that of group 2 and 3 was not significant.

Result from Figure 30 shows that there was an increase in WBC level in group 2 (7.02±0.07), 3 (7.22±0.10), and 4 (7.77±0.26) when compared to group 1 (6.45±0.15), but the increase was significant in group 3 and 4 while that of group 2 was not significant.

For platelet count (Figure 31), there was an increase in group 2 (257.50±3.22), 3(274.25±4.97), and 4 (286.25±5.54), when compared to group 1 (236.25±2.39), but the increase was significant across the treatment groups.

- **Body Weight**

The effect of ethyl acetate fraction of *B. pubescens* on Body weight is presented in Figure 32. All data were analyzed using Student dependent T-test and data were considered significant at $p<0.05$. *$p<0.05$ shows that it was significant, and **$p<0.05$ shows that it was more significant.

Results presented in Figure 32 shows that there was an increase in the body weight in group 1 when comparing the initial weight (145.00 ±2.88) and final weight (195.00±13.22), but it was significant. For group 2, there was a significant increase in the body weight when comparing the Initial (170.00±4.08) and Final (227.50±11.08) body weight. For group 3, there was a significant increase in the body weight when comparing the Initial (165.00±6.45) and Final (212.50±6.29) body weight. For group 4, there was a significant increase in the body weight when comparing the Initial (177.50±4.78) and Final (235.00±6.45) body weight.

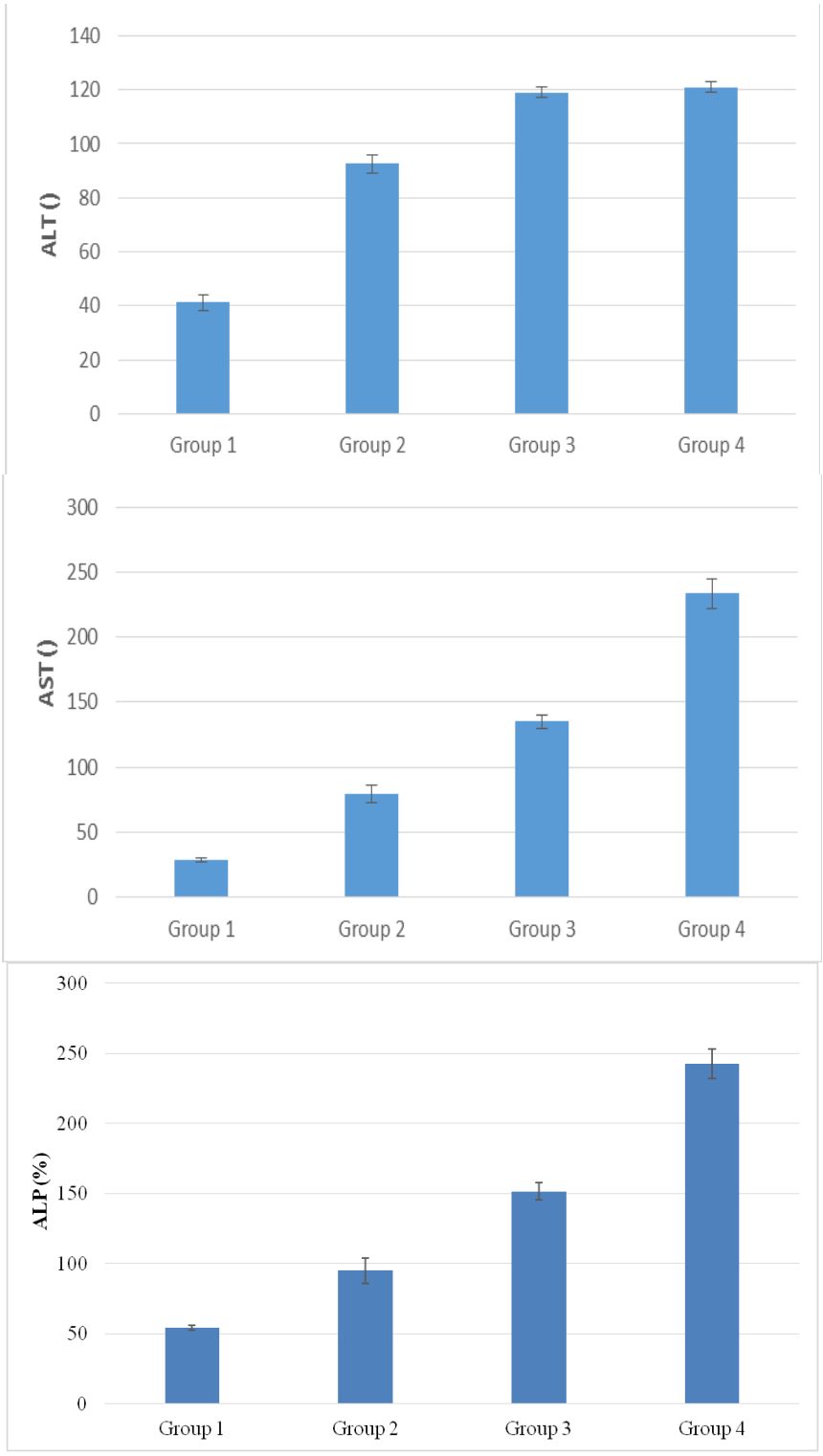


Figure 26: The effect of ethyl acetate fraction of *B. pubescens* on ALT, AST and ALP levels

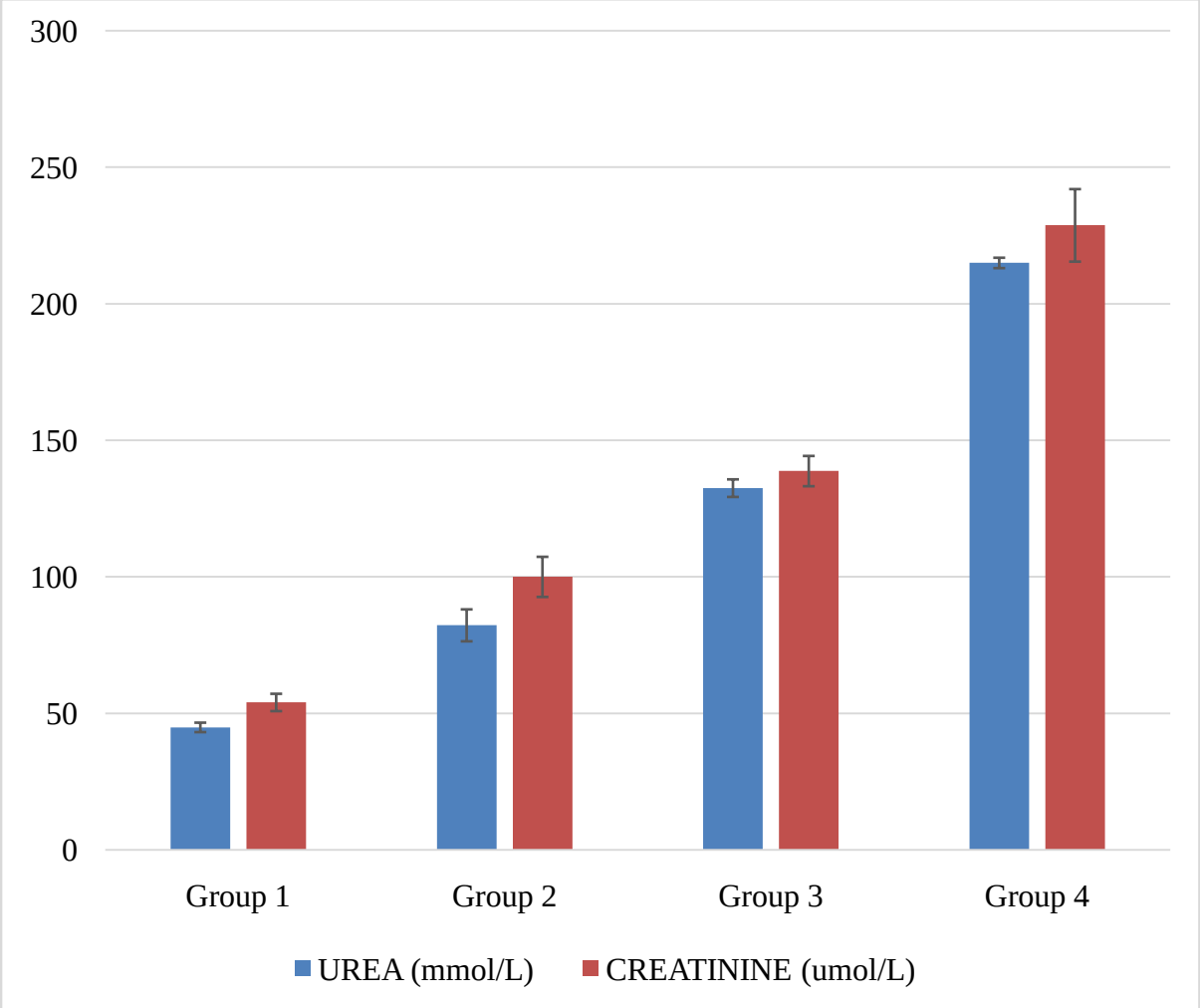


Figure 27: The effect of ethyl acetate fraction of *B. pubescens* on urea and creatinine level

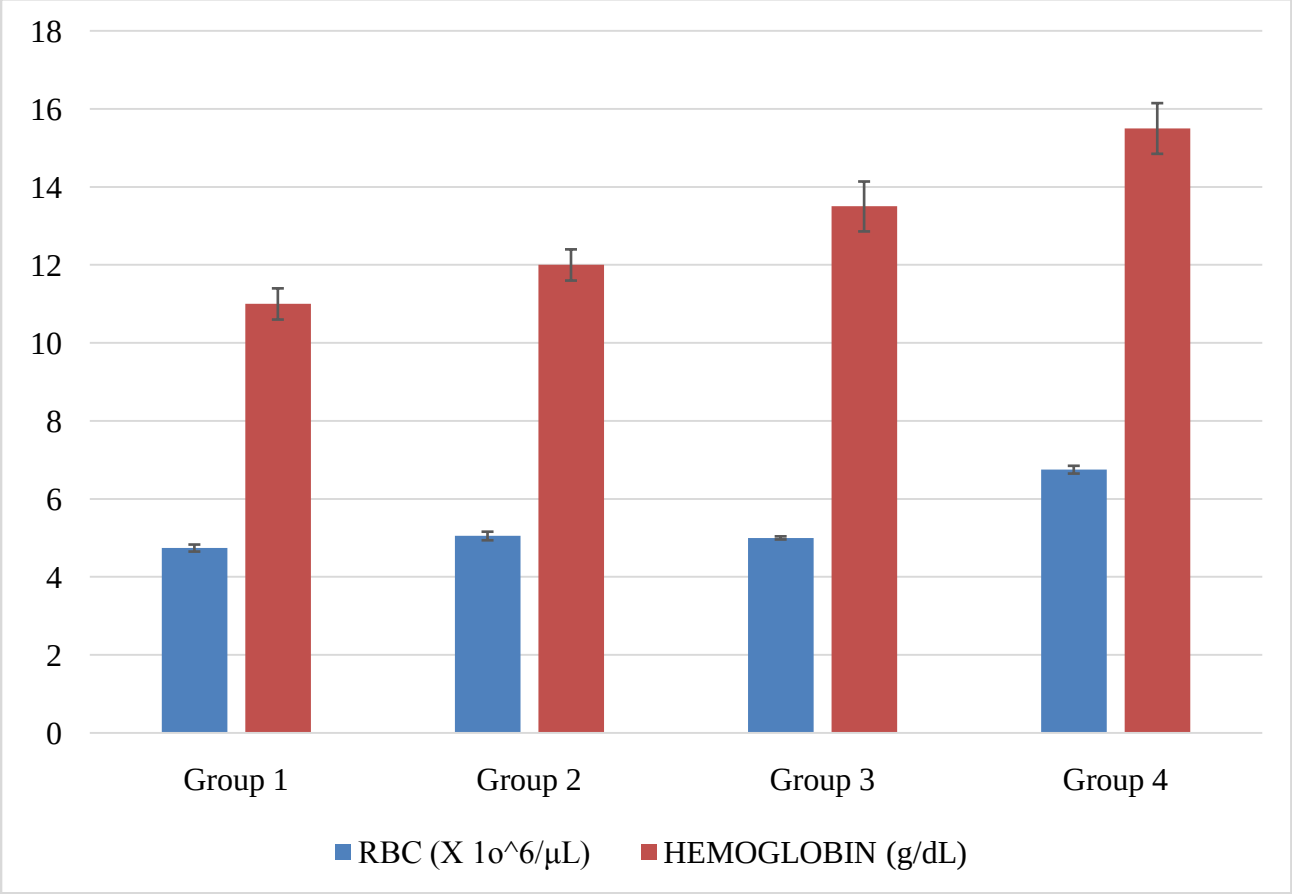


Figure 28: The effect of ethyl acetate fraction of *B. pubescens* on RBC and hemoglobin level

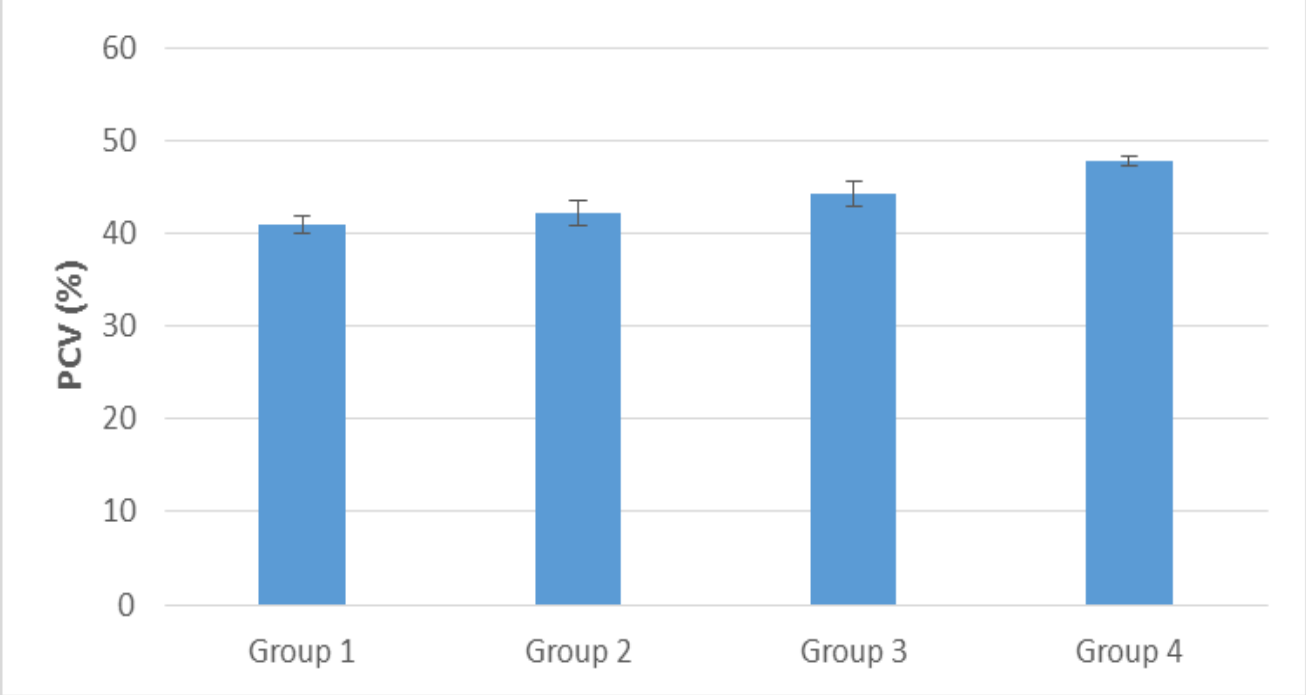


Figure 29: The effect of Ethyl acetate fraction of *B. pubescens* on PCV level

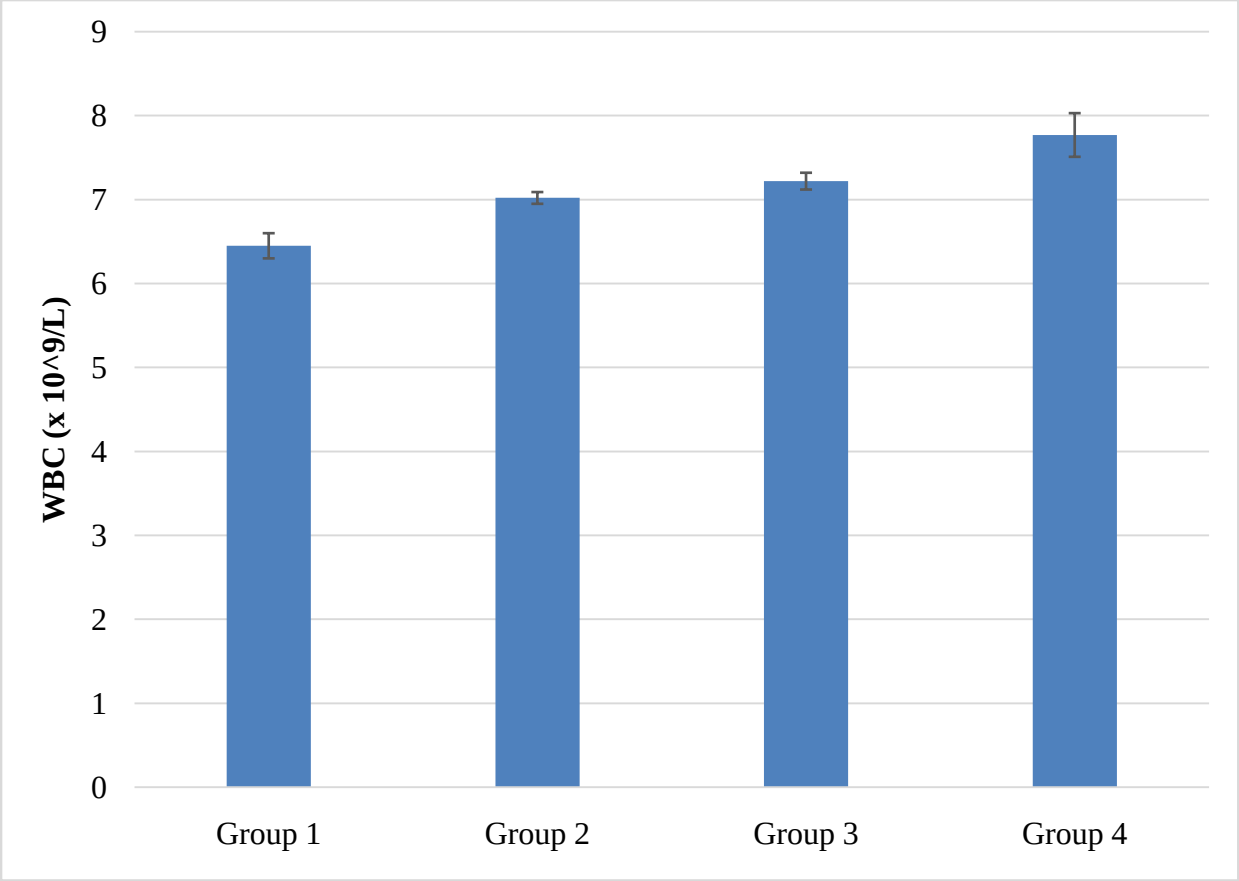


Figure 30: The effect of ethyl acetate fraction of *B. pubescens* on WBC level

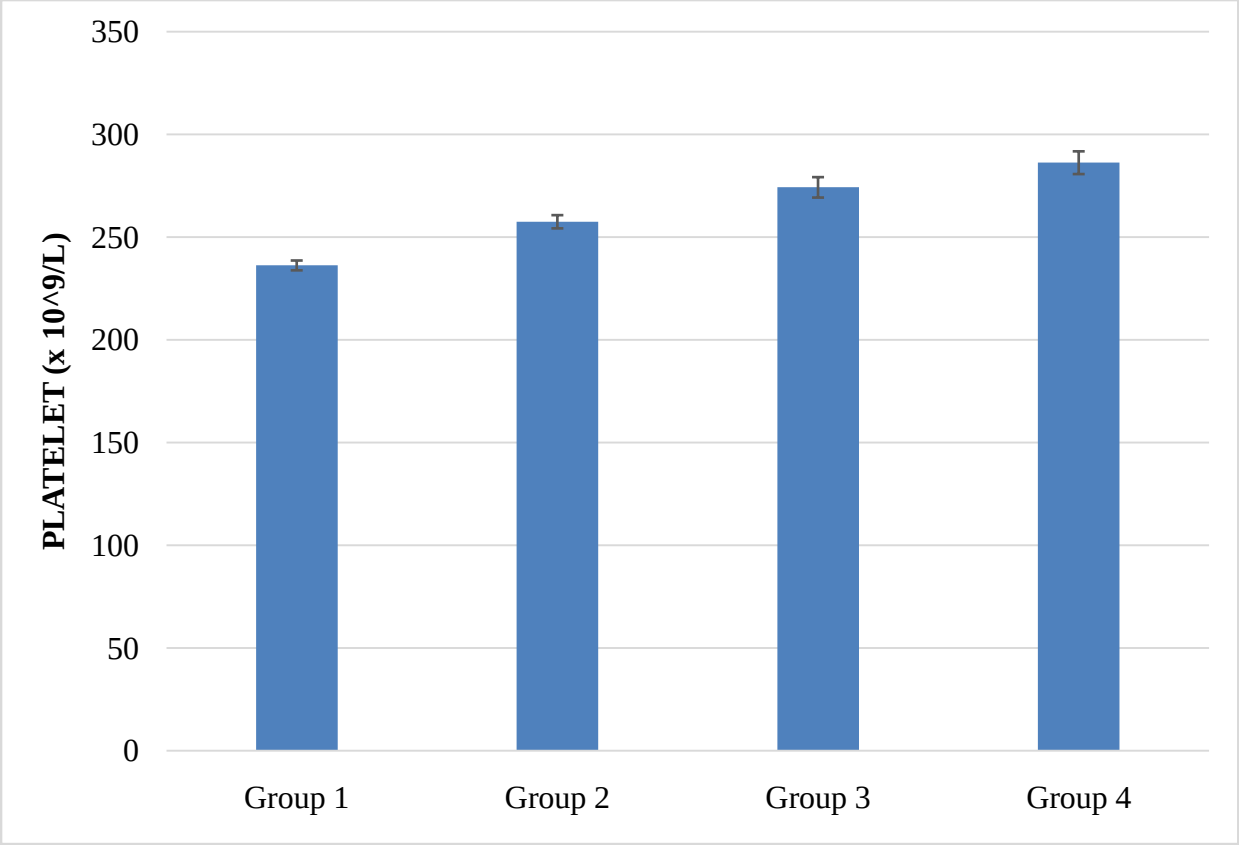


Figure 31: Effect of ethyl acetate fraction of *B. pubescens* on platelet count level

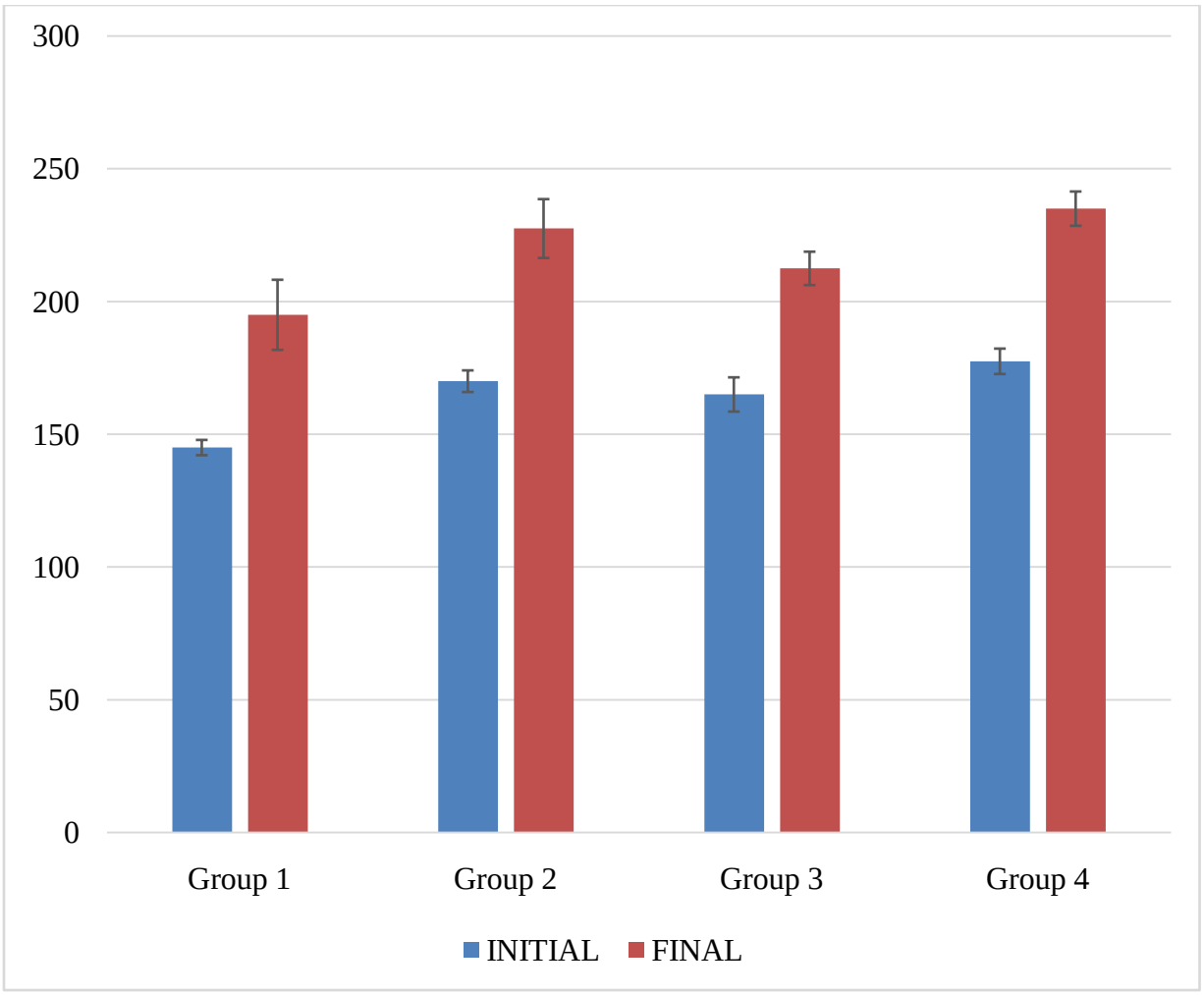


Fig ure 32: The effect of ethyl acetate fraction of *B. pubescens* on body weight

4.7.1 Histopathological findings

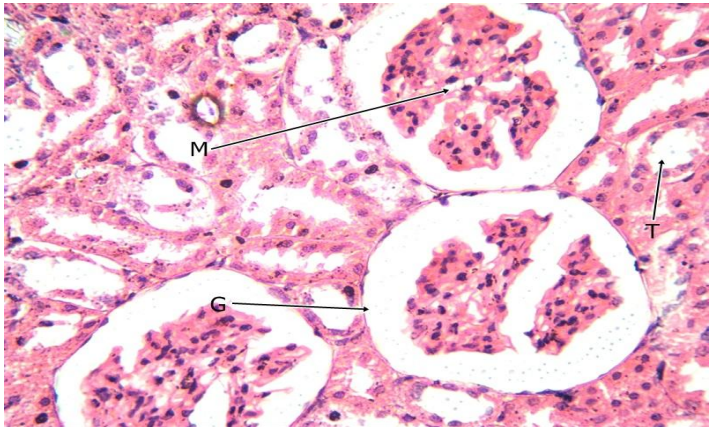
Photomicrographs showing the histology of the normal and treated cells of the kidney are presented in Figure 33 (A-D).

In Figure 33 (A), the photomicrograph shows the normal histology of the kidney. The glomeruli were evenly distributed and of similar size, density of up to 32 per HPF with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable.

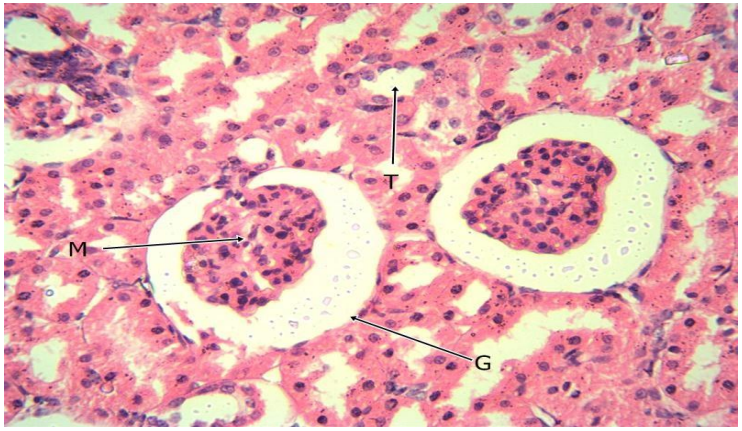
Figure 33 (B) is a photomicrograph showing the glomeruli of similar size, density of up to 30 per HPF with mild mesangial hypercellularity consisting of proliferating mesangial cells. There are few open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. The interstitium is thin and there is mild interstitial.

In Figure 33 (C), the photomicrographs indicates that the glomeruli are of mildly variable size, density of up to 33 per HPF with normal mesangial cellularity. There are many open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. The interstitium is thin and there is mild interstitial.

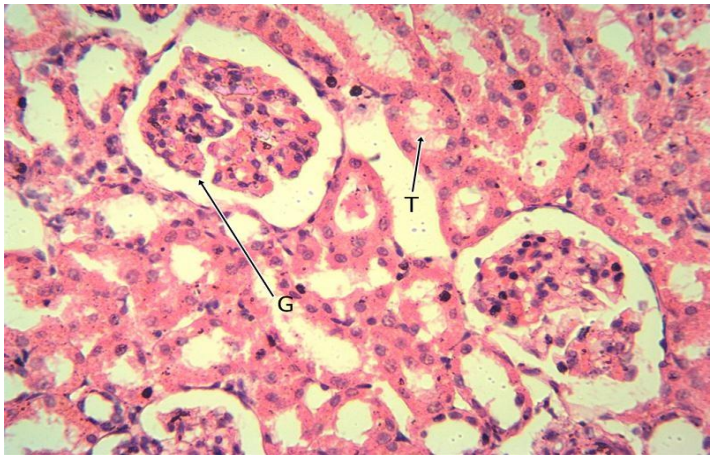
In Figure 33 (D), the photomicrographs shows the glomeruli are of similar size, density of up to 30 per HPF with mild mesangial hypercellularity consisting of proliferating mesangial cells. There are few open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. The interstitium is thin and there is mild interstitial.



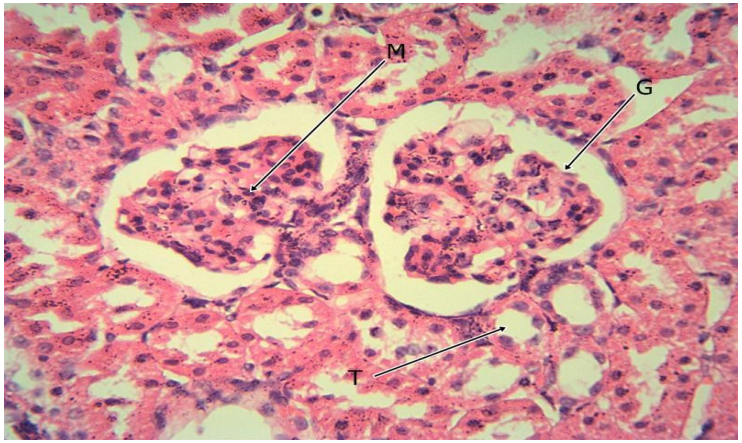
A: Photomicrograph of the kidney cells
(Group 1; Control)



B: Photomicrograph of the kidney cells
(Group 2; 250mg/Kg of *B. pubescens* ethyl acetate fraction)



C: Photomicrograph of the kidney cells
(Group 3; 500mg/Kg of *B. pubescens* ethyl acetate fraction)



D: Photomicrograph of the kidney cells
(Group 4; 1000mg/Kg of *B. pubescens* ethyl acetate fraction)

Figure 33: Photomicrographs showing the histology of the normal and treated cells of the kidney. Stained by H&E Technique. X400.Where G= Glomeruli, M= Mesangium, T= Tubule.

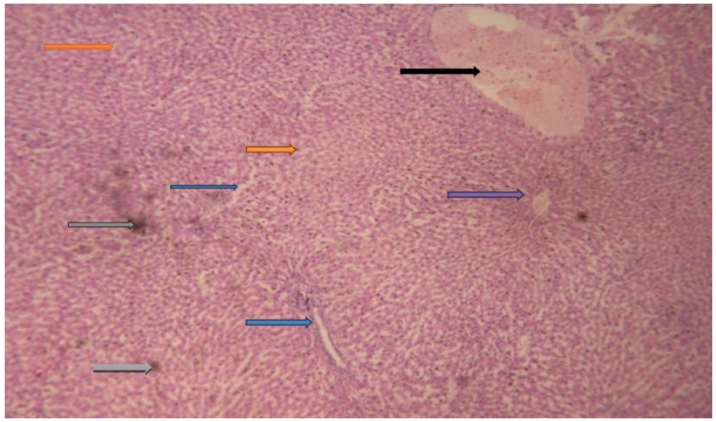
Also, photomicrographs showing the histology of the normal and treated cells of the liver are presented in Figure 34 (A-D)

In Figure 34 (A), the photomicrograph shows a well preserved liver architecture. The centrallobular vein marked by (black arrow), portal triads evenly spaced marked by (yellow arrow), liver sinusoids (marked by blue arrows), hepatocytes cells (marked by green arrow), erythrocyte cells (marked by orange arrow) and nuclei (marked by grey arrows) with evidence of free anastomsing peri-portal limiting plates.

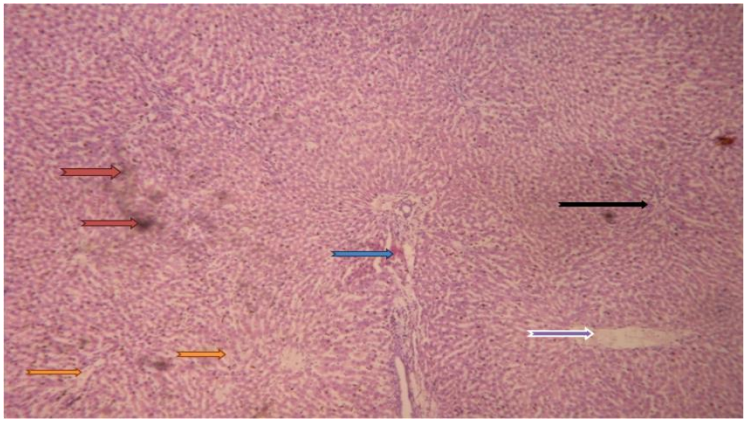
In Figure 34 (B), the central vein marked by (Yellow arrow) showed mild congested blood vessels, the portal triad marked by (Black arrow) shrinkage of cells, proliferation of liver cells with blood filled liver sinusoids (marked by blue arrow), Mild lobular inflammation marked by (Green arrows), Mild Necrosis marked by (Orange arrows).

Figure 34 (C) displays a well preserved liverarchitecture. The portal are evenly spaced around the central vein marked by (Blue arrows), the central vein showing mild congested blood vessels marked by (Black Arrows), and Occasional lobular inflammation without confluent necrosis marked by (White arrows).

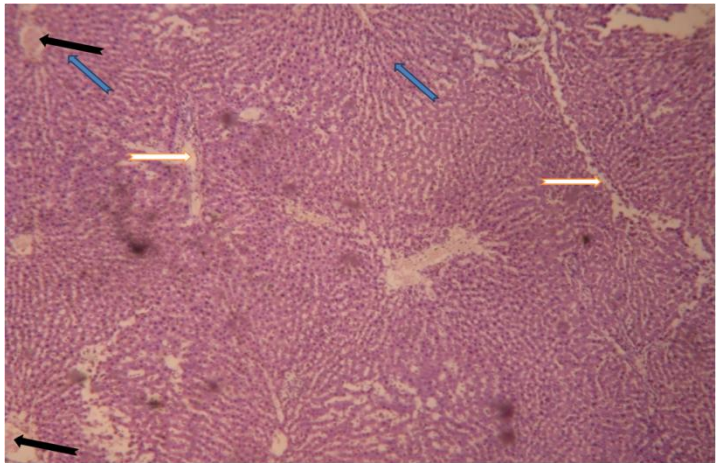
In Figure 34 (D), the central vein with portal triads evenly spaced with mild peri-portal inflammation marked by (Blue arrows), Mild necrosis marked by (Black arrows), No lobular inflammation marked by (White arrows).



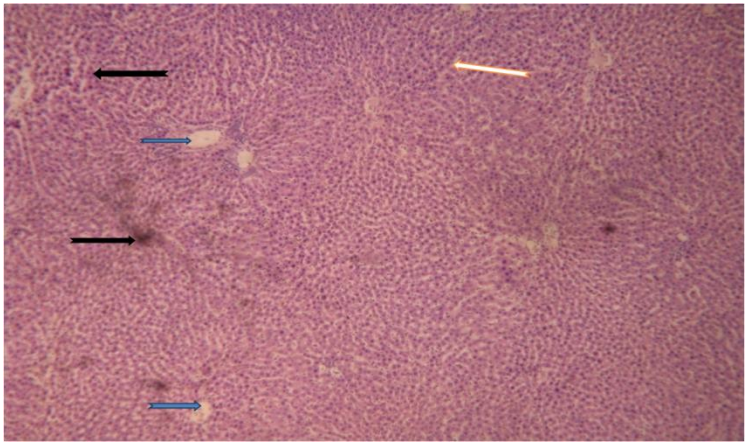
A: Photomicrograph of the liver cells
(Group 1, control)



B: Photomicrograph of the liver cells
(Group 2, 250mg/Kg of *B. pubescens* ethyl acetate fraction)



C: Photomicrograph of the liver cells
(Group 3; 500mg/Kg of *B. pubescens* ethyl acetate fraction)



D: Photomicrograph of the liver cells
(Group 4; 1000mg/Kg of *B. pubescens* ethyl acetate fraction)

Figure 34: Photomicrographs showing the histology of the normal and treated cells of the liver. Stained by H & E (X 100)

4.8 ANTIMALARIAL ACTIVITIES OF SUB-FRACTIONS O*F B. PUBESCENS* ETHYL ACETATE FRACTION

Results of the antimalarial activities of sub-fractions o*f B. pubescens* ethyl acetate fraction are presented in Figure 35. Subfraction D7 exhibited the best activity followed by subfractions D2, D5 and D7.

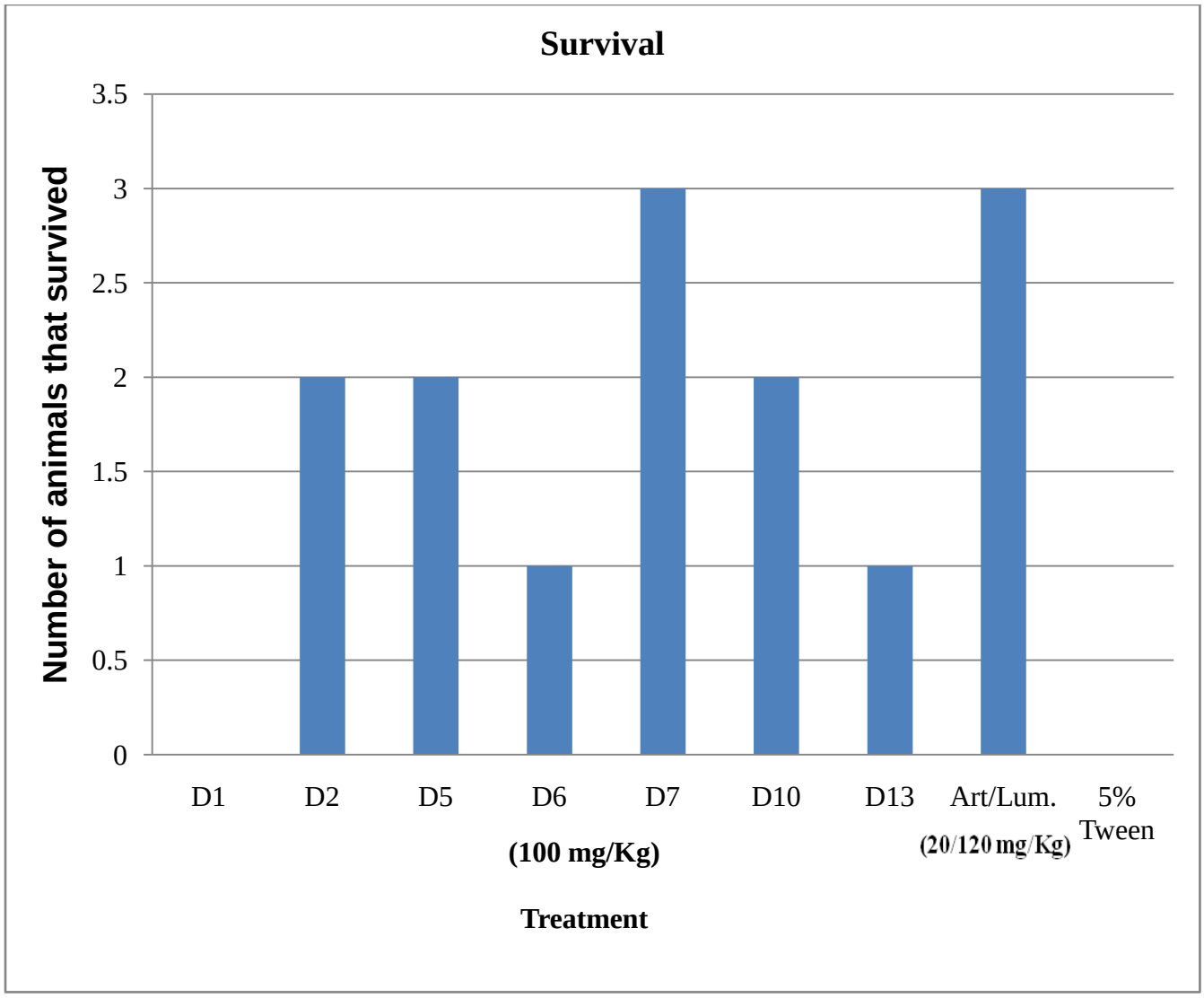


Figure 35: *In vivo* antimalarial Activities of sub-fractions o*f B. pubescens* ethyl acetate fraction

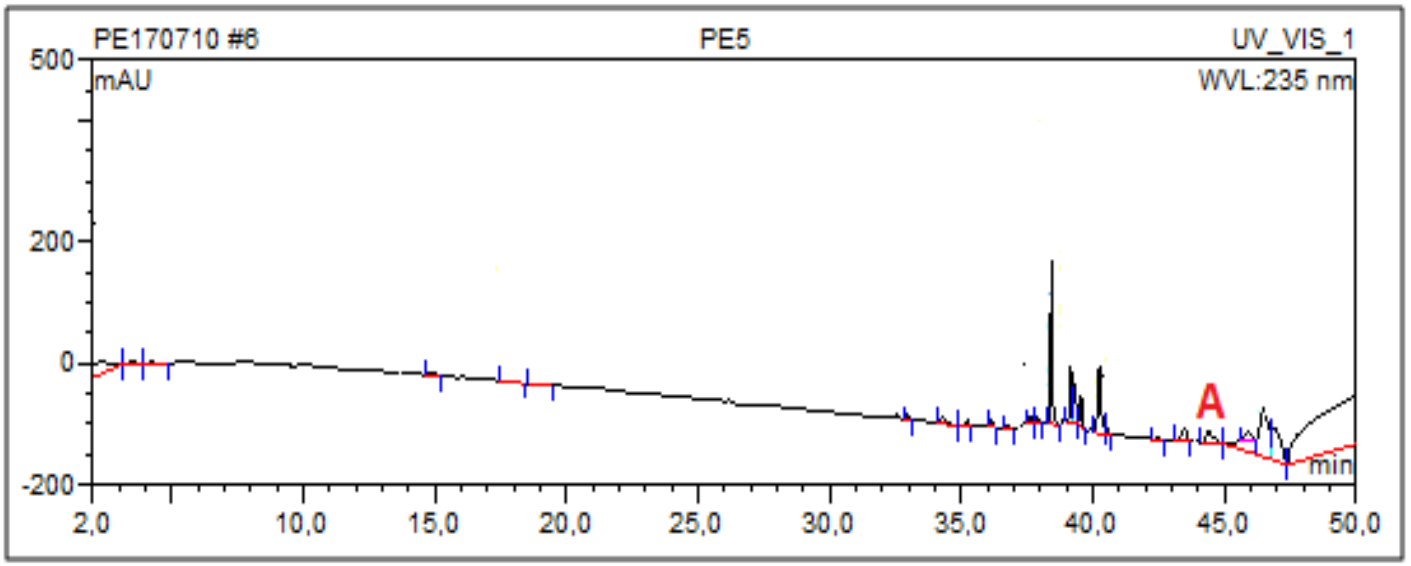
4.9 HPLC-DAD ANALYSIS OF THE SUBFRACTIONS OF *B. PUBESCENS*

Results of the HPLC-DAD analysis of *B. pubescens* Sub-fractions are presented in Table 15. Also, the HPLC chromatograms of the subfractions of *B. pubescens* ethyl acetate fraction, showing the detected compounds are shown in Figures 36-42. The following compounds were detected cytosporin D, cerebroside, palitantin, aureonitol, septicine, and citreodrimene F. The UV-spectra and structures of the detected compounds are shown in Figures 43-49.

Table 15: Result of HPLC-DAD Analysis of *B. pubescens* Sub-fractions

Samples	Compounds detected	Retention time	UV max	Chemical formula
Subfraction 4	Palitantin	44.39	231.2	$C_{14}H_{22}O_4$
Subfraction 5	Aureonitol	34.28	233.1	$C_{13}H_{18}O_2$
	Cerebroside	35.21	233.2	
Subfraction 6	Cytosporin D	22.87	238.4	$C_{19}H_{30}O_5$
	Palitantin	35.59	277.2	$C_{14}H_{22}O_4$
Subfraction 7	No hit	-	-	-
Subfraction 8	Cerebroside	34.37	233.2	$C_{41}H_{77}NO_9$
	Aureonitol	35.26	233.2	$C_{13}H_{18}O_2$
Subfraction 9	Palitantin	34.32	231.5	$C_{14}H_{22}O_4$
	Aureonitol	35.22	233.1	$C_{13}H_{18}O_2$
Subfraction 10	No hit	-	-	-
Subfraction 11	No hit	-	-	-
Subfraction 12	No hit	-	-	-
Subfraction 13	No hit	-	-	-
Subfraction 14	Septicine	16.37	207	$C_{24}H_{29}NO_4$
Subfraction 15	Citreodrimene F	3.69	222	$C_{15}H_{22}O_4$
	Septicine	16.39	206.8	$C_{24}H_{29}NO_4$
Subfraction 16	No hit	-	-	-
Subfraction 17	No hit	-	-	-
Subfraction 18	No hit	-	-	-
Subfraction 19	No hit	-	-	-

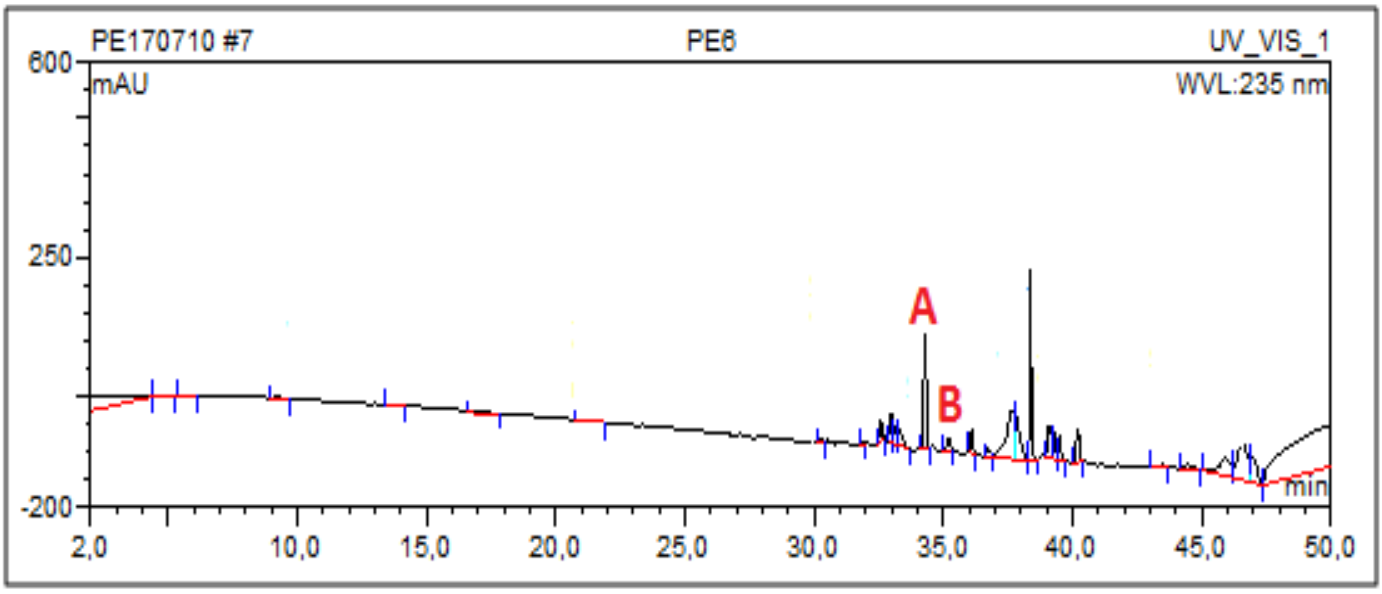
SUBFRACTION 4



A: Palitantin

Figure 36: HPLC chromatogram of Subfraction 4 of *B. pubescens* ethyl acetate fraction, showing the bioactive compound palitantin

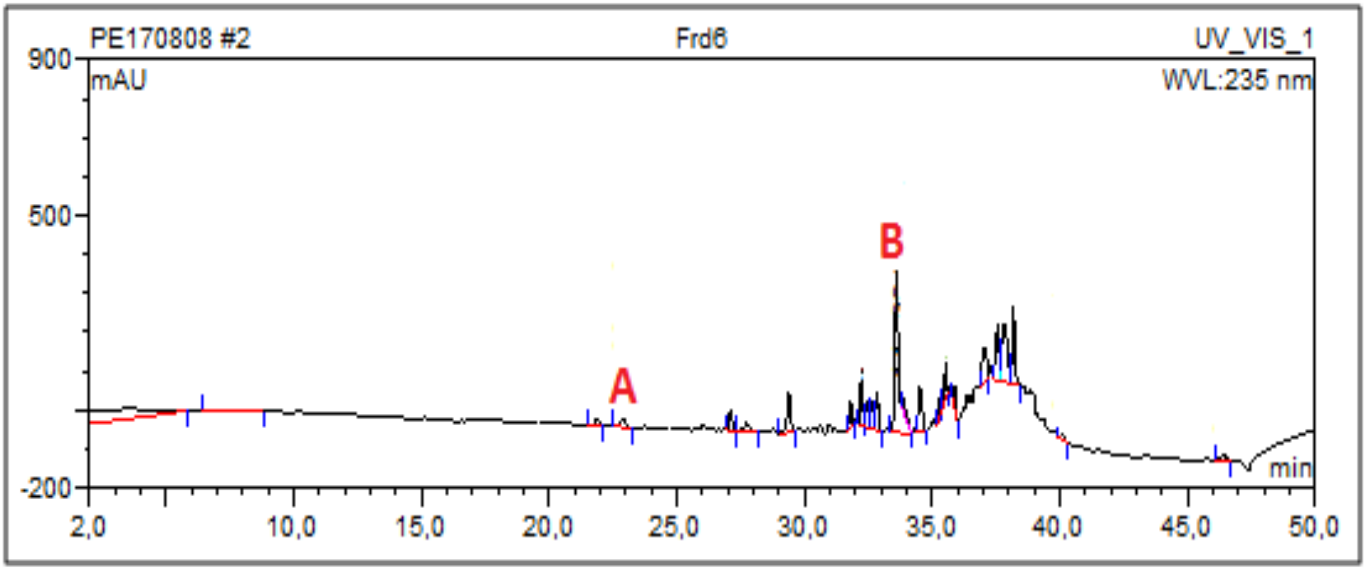
SUBFRACTION 5



A:Aureonitol
B: Cerebroside

Figure 37: HPLC chromatogram of Subfraction 5 of *B. pubescens* ethyl acetate fraction, showing the bioactive compounds aureonitol and cerebroside

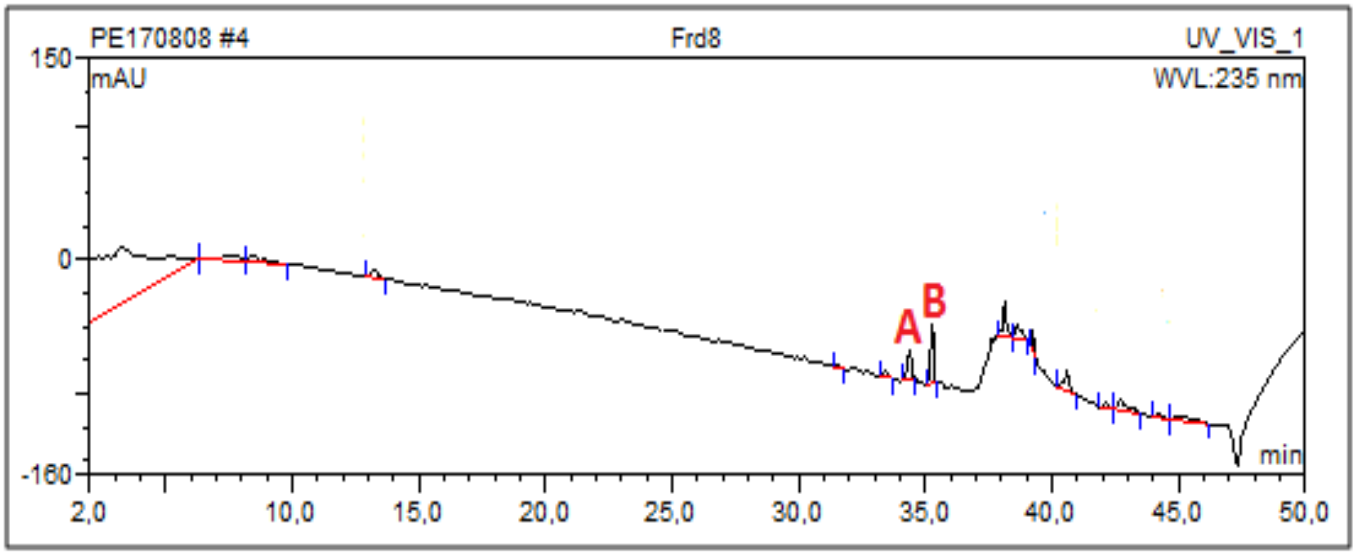
SUBFRACTION 6



A: Cytosporin D
B: Palitantin

Figure 38: HPLC chromatogram of Subfraction 6 of *B. pubescens* ethyl acetate fraction, showing the bioactive compounds cytosporin D and palitantin

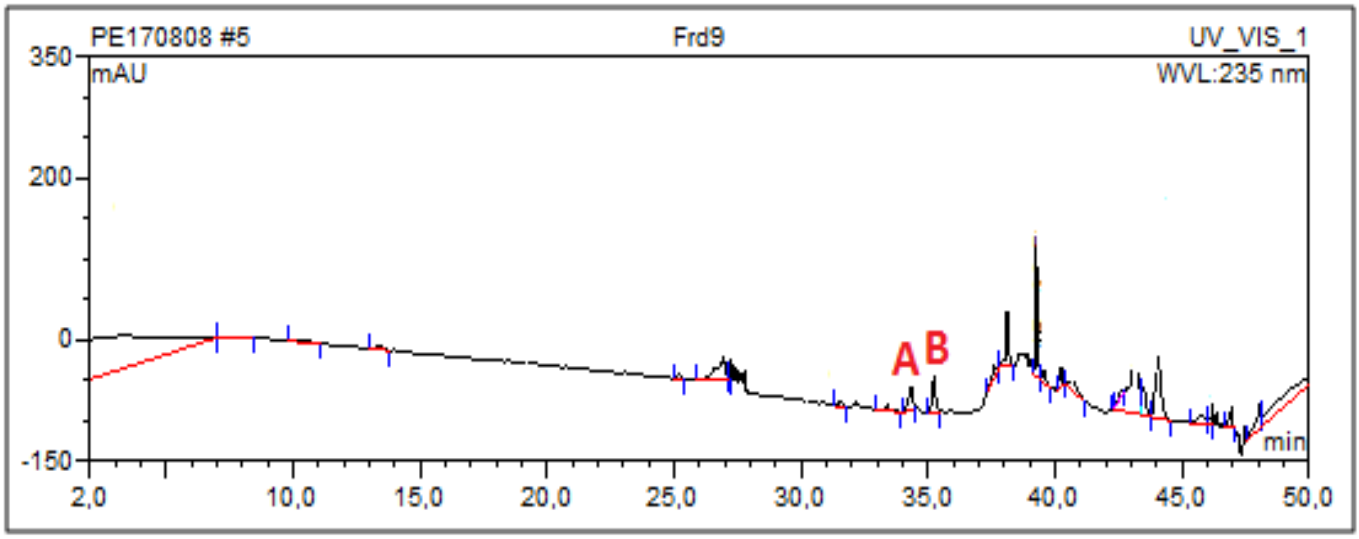
SUBFRACTION 8



A: Cerebroside
B:Aureonitol

Figure 39: HPLC chromatogram of Subfraction 8 of *B. pubescens* ethyl acetate fraction, showing the bioactive compounds cerebroside and aureonitol

SUBFRACTION 9

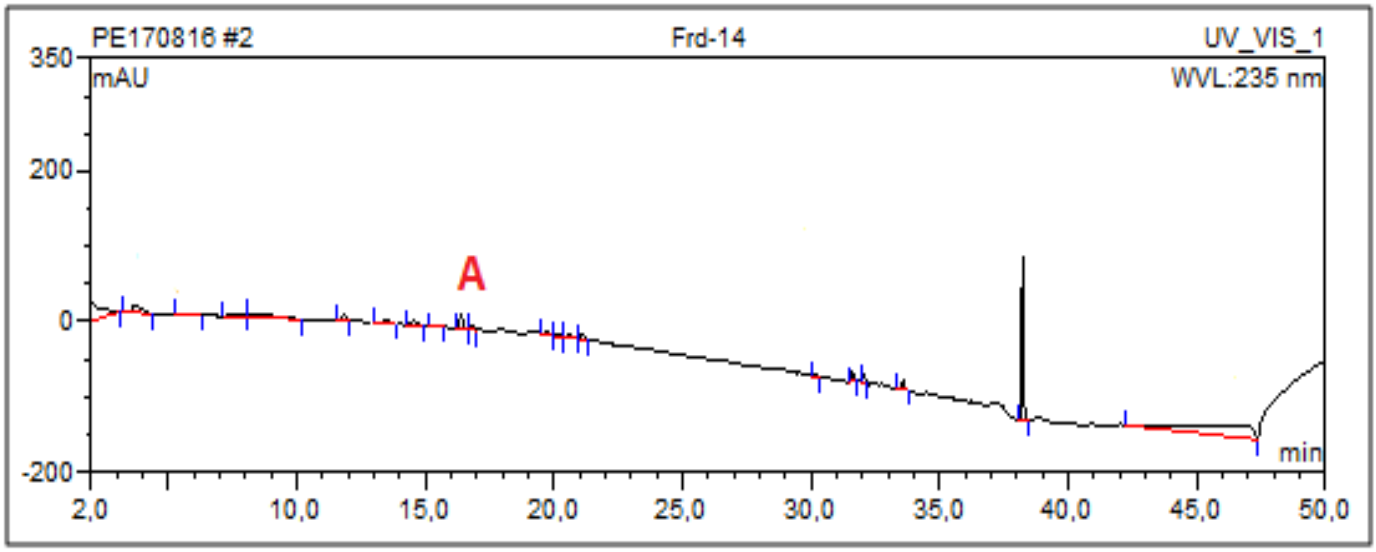


A: Palitantin

B: Aureonitol

Figure 40: HPLC chromatogram of Subfraction 9 of *B. pubescens* ethyl acetate fraction, showing the bioactive compounds palitantin and aureonitol

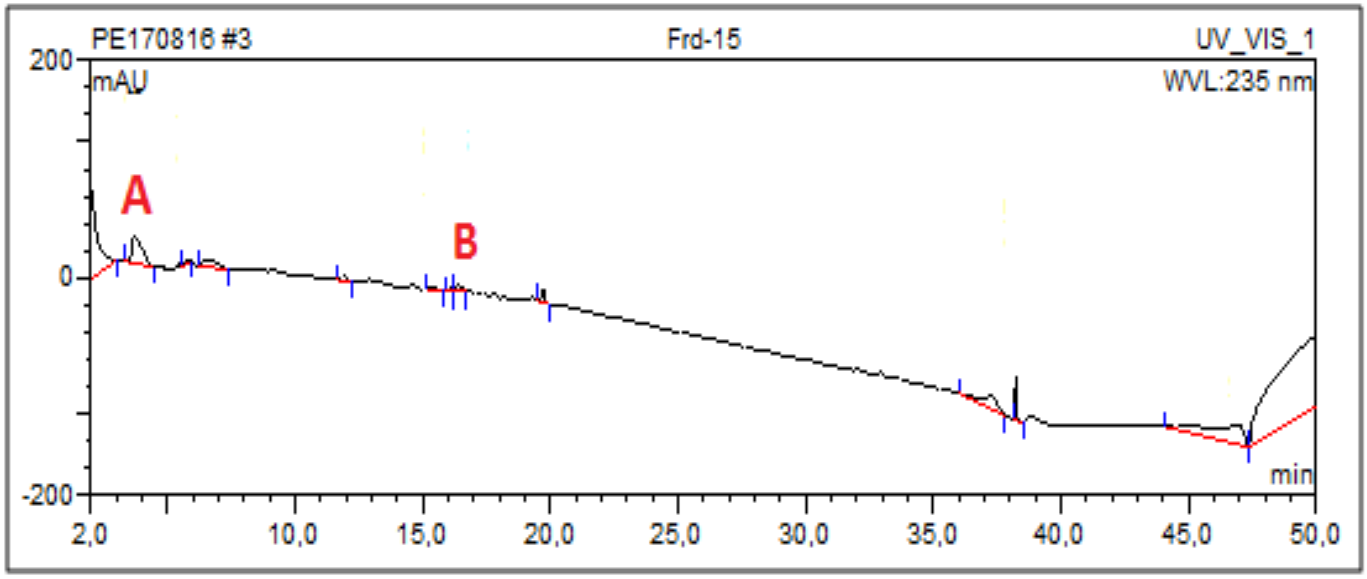
SUBFRACTION 14



A: Septicine

Figure 41: HPLC chromatogram of Subfraction 14 of *B. pubescens* ethyl acetate fraction, showing the bioactive compound septicine

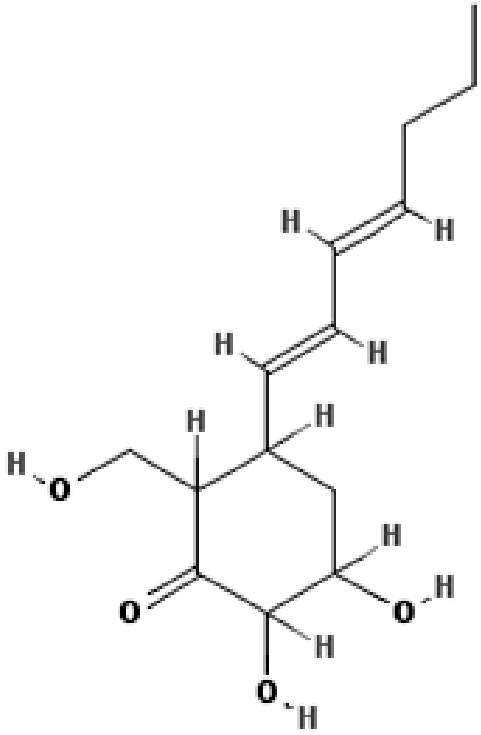
SUBFRACTION 15



A: Citreodrimene F
B: Septicine

Figure 42: HPLC chromatogram of Subfraction 15 of *B. pubescens* ethyl acetate fraction, showing the bioactive compounds citreodrimene F and septicine

A. Palitantin



Chemical formula: $C_{14}H_{22}O_4$

Molecular mass: 254.326 g/mol

UV max: 231 nm

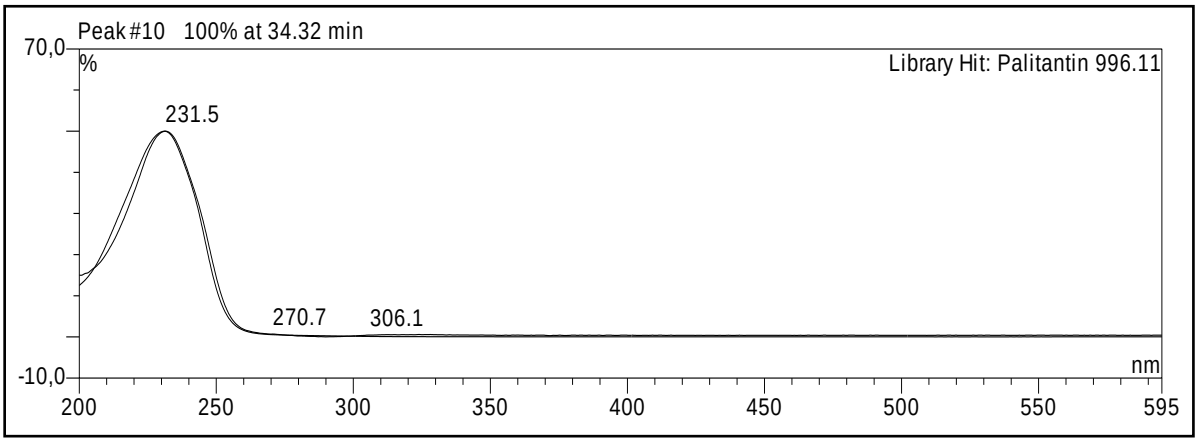
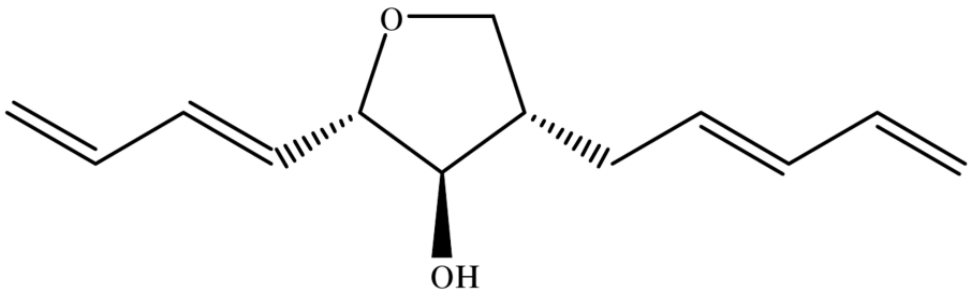


Figure 43: Structure, Chemical formula, Molar mass and UV-spectrum of Palitantin

B. Aureonitol



Chemical formula: $C_{13}H_{18}O_2$

Molecular mass: 206.28 g/mol

UV max: 233.1 nm

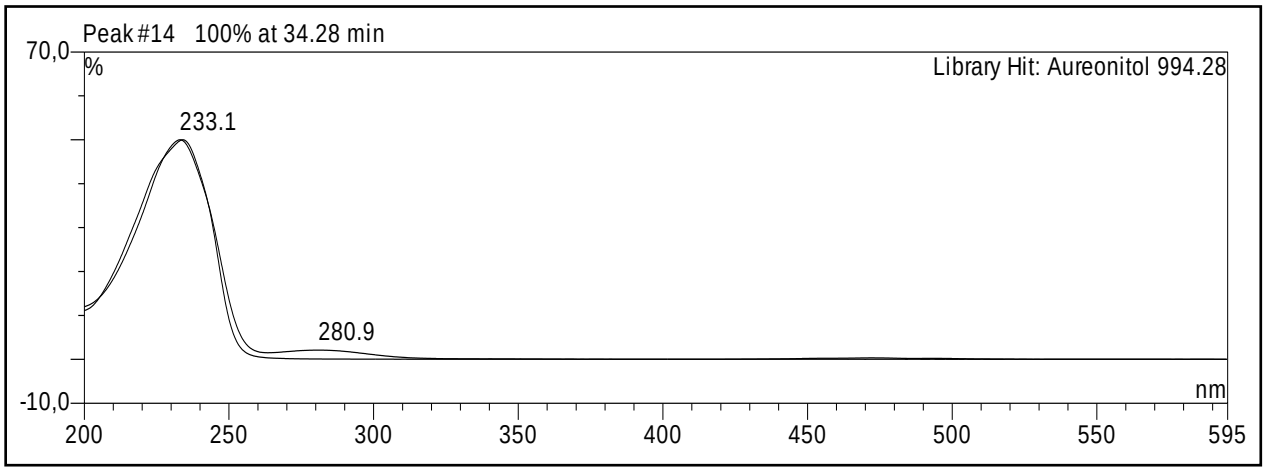
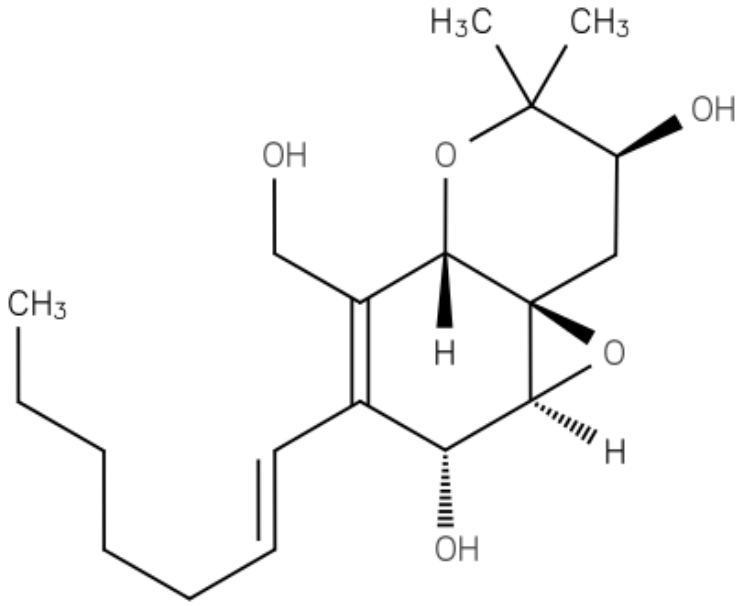


Figure 44: Structure, Chemical formula, Molar mass and UV-spectrum of Aureonitol

C. Cytosporin D



Chemical formula: $C_{19}H_{30}O_5$

Molecular mass: 338.4 g/mol

UV max: 238 nm

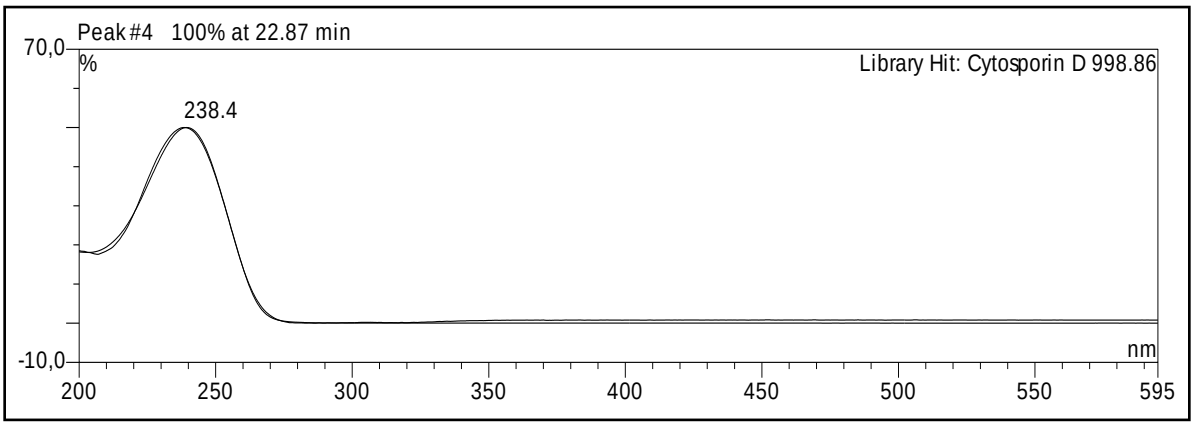
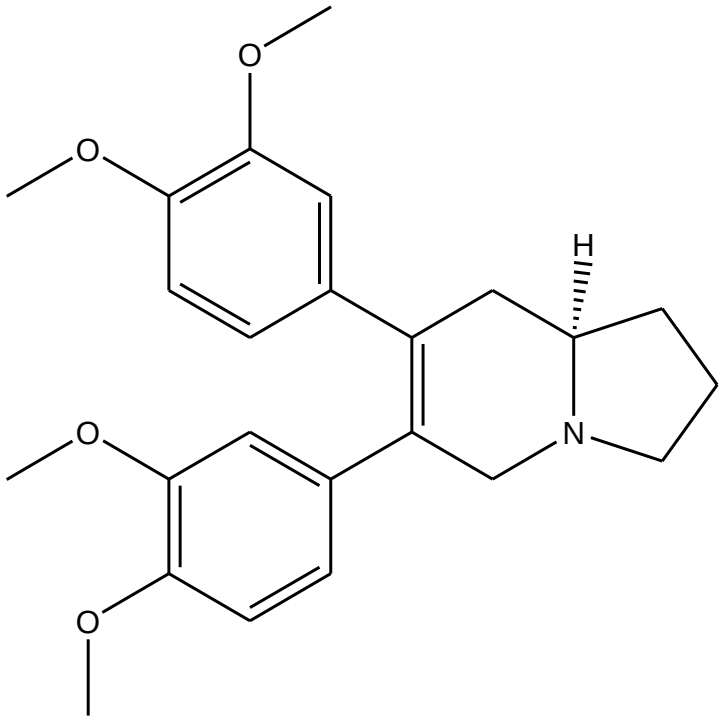


Figure 45: Structure, Chemical formula, Molar mass and UV-spectrum of Cytosporin D

D. Septicine



Chemical formula: $C_{24}H_{29}NO_4$

Molecular mass: 395.499 g/mol

UV max: 207 nm

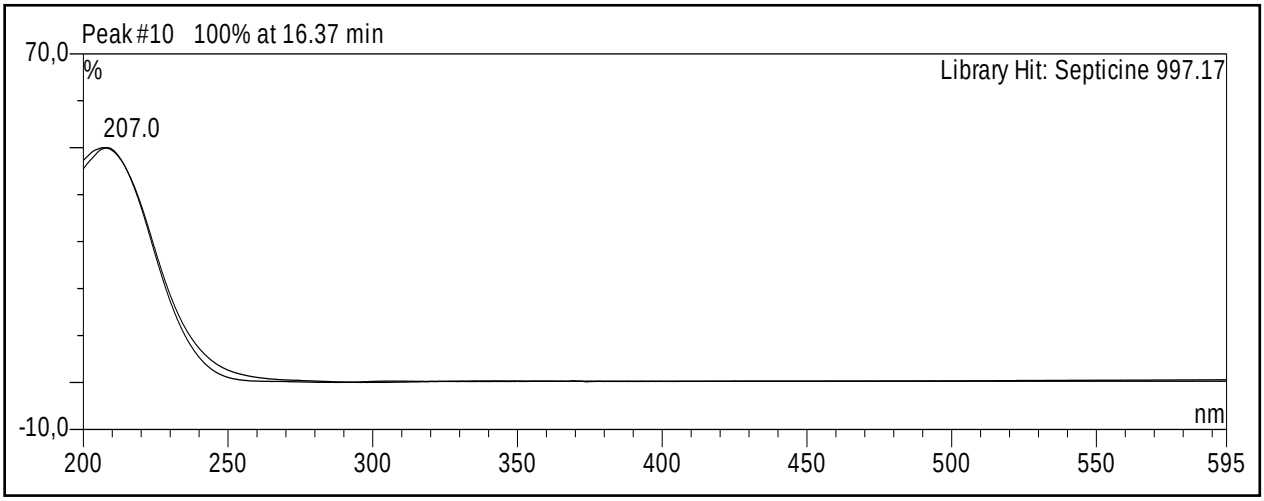
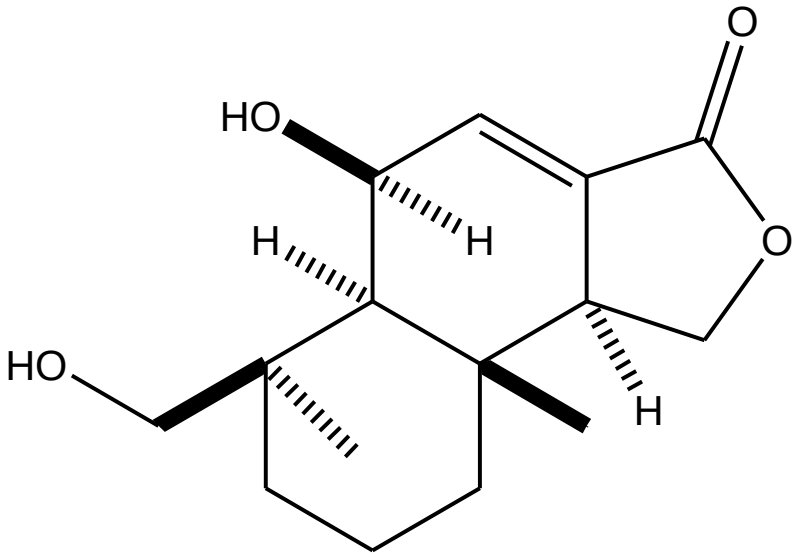


Figure 46: Structure, Chemical formula, Molar mass and UV-spectrum of Septicine

E. Citreodrimene F



Chemical Formula: $C_{15}H_{22}O_4$
Molecular mass: 266 g/mol
UV max: 222 nm

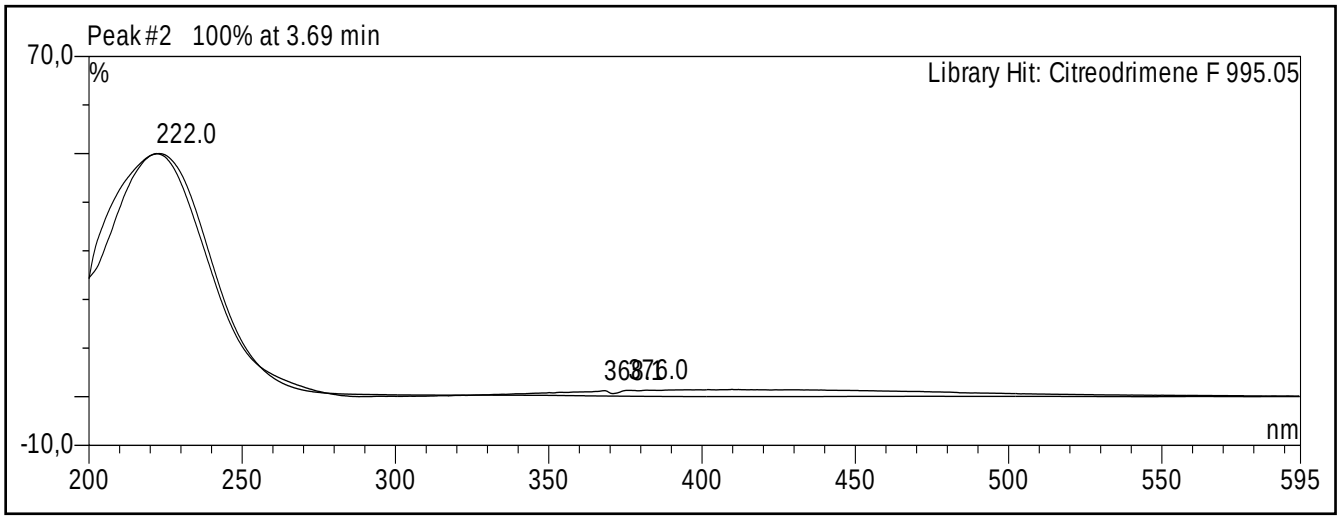
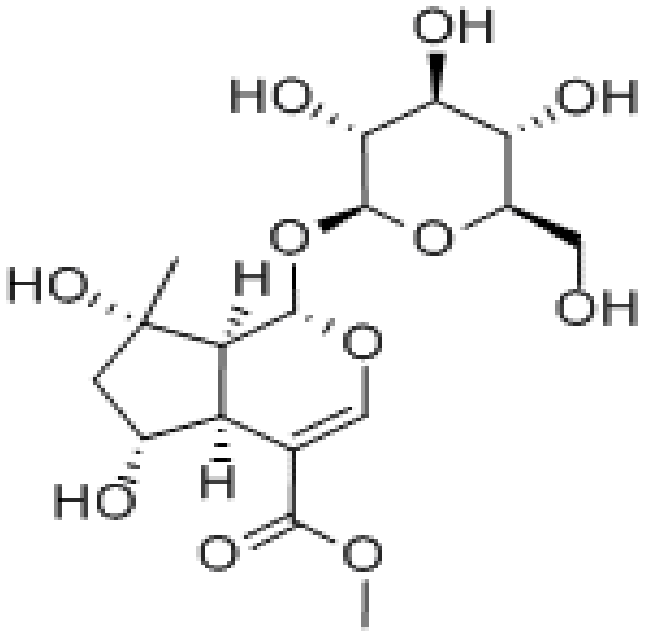


Figure 47: Structure, Chemical formula, Molar mass and UV-spectrum of Citreodrimene F

F. Shanzhiside methyl ester



Chemical Formula: $C_{17}H_{26}O_{11}$

Molecular Weight: 406.38 g/mol

UV max: 236.4 nm

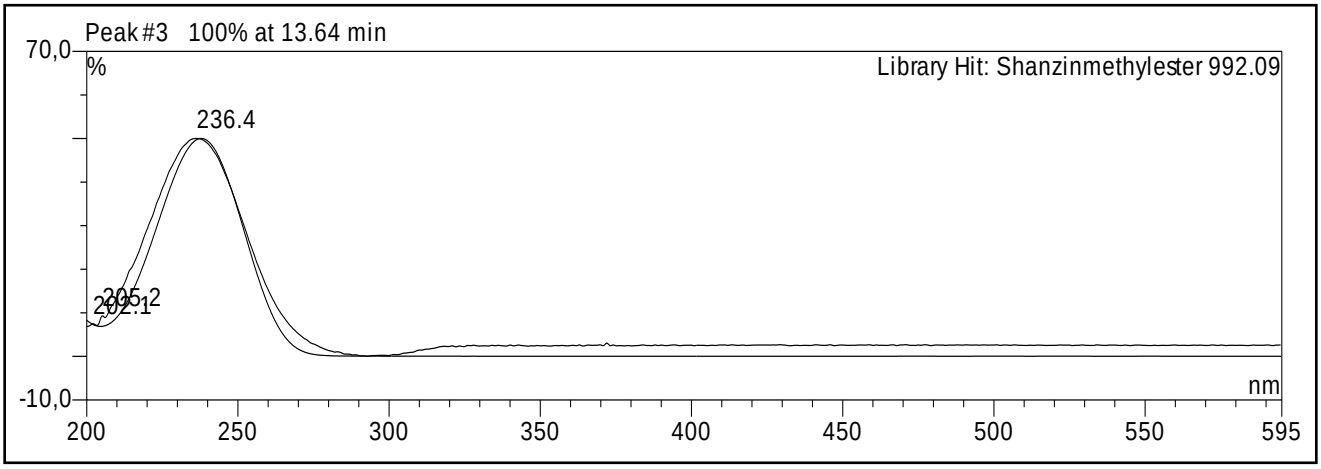
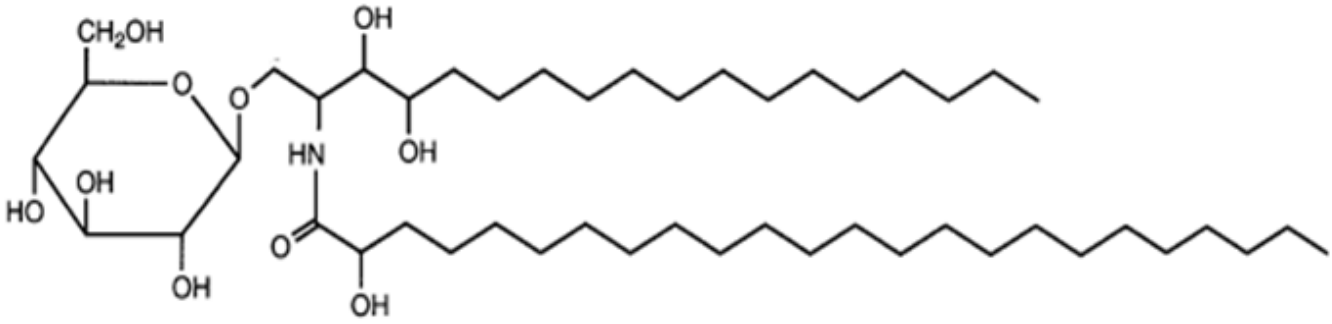


Figure 48: Structure, Chemical formula, Molar mass and UV-spectrum of Shanzhiside methyl ester

G. Cerebroside



Chemical Formula: $C_{41}H_{77}NO_9$

Chemical Formula: 728 g/mol

UV max: 233.4 nm

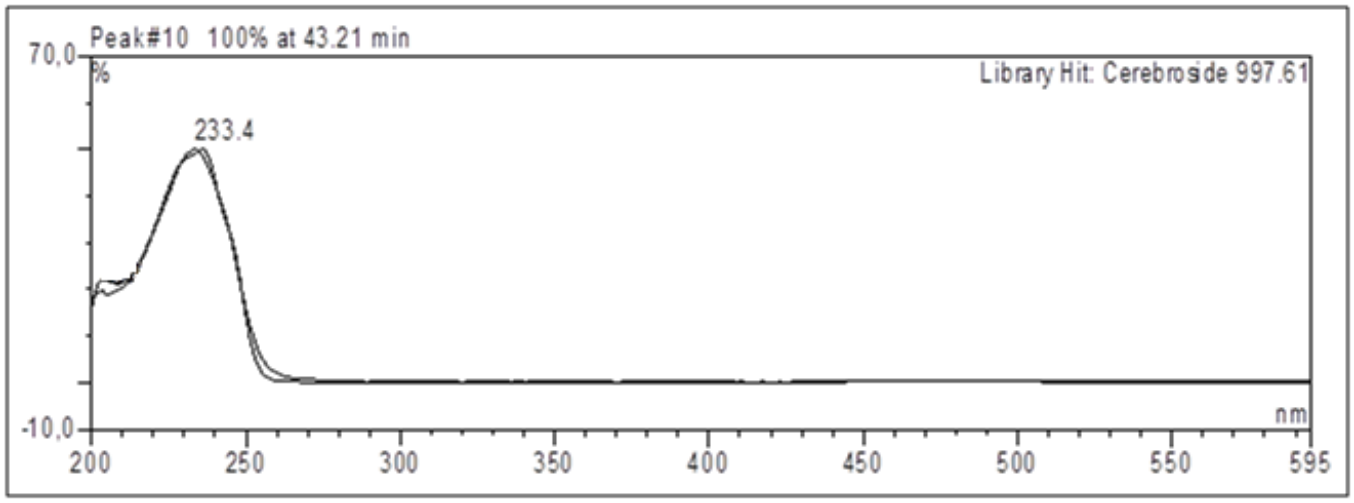


Figure 49: Structure, Chemical formula, Molar mass and UV-spectrum of Cerebroside

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Malaria poses a major health problem to more than half of the world's population. However, with reports of decreased sensitivity to current first line drugs such as arthemether/lumefantrine, an artemisinin-based combination treatment (ACT) *(*Kayamo *et al.*, 2011); there arises the need for new drugs to combat this problem. This has led to increase in research for investigation of a new alternative source for treatment of malaria including medicinal plant (Oliveira et al., 2009).

Plant materials remain an important source of medicine in the fight against malaria, since widely used anti-malarial drugs such as quinine and artemisinin were isolated from plants, and because of the increased resistance to and lack of accessibility to existing affordable synthetic drugs (Aderounmu, 2007).

In line with this idea, the following plants were screened for their antimalarial activities – *K. africana, B. pubescens, M. lucida, S. scabrida, B. coriaceae, R. vomitoria, N. latifolia, A. djalonensis and M. oleiferae.* These plants are all used by the people of Ogidi in Anambra state of Nigeria in the traditional treatment of malaria. From the preliminary studies, they all have varying degrees of anti-malarial properties. At 100 mg/Kg each of them had the following percentage curative acitivities – *K. africana* 90%, *B. pubescens* 97%s, *M. lucida* 85%, *S. scabrida* 78%, *B. coriaceae* 78%, *R. vomitoria* 92%, *N. latifolia* 81%, *A. djalonensis* 54% and *M. oleifera* 58%. But *B. pubescens* showed the highest potency, followed by *K. africana.*

B. pubescens was selected for further investigations because of its promising activity and safety at (LD_{50}) 5000/kg. Also, bioassay-guided-extraction of *B. pubescens* was performed using *n*-hexane, ethyl acetate, and butanol to obtain various fractions of the plant extract. These were screened against malarial parasites, and the ethyl acetate fraction was found to be the most effective giving almost 100% cure at 100 mg/Kg.

The ethyl acetate fraction, being the most promising, was subjected to various scientific tests.

Chronic toxicity studies on the ethyl acetate fraction of *B. pubescens* indicated that the plant is relatively safe at the therapeutic dose. The toxicity of the plant was shown to be dose dependent. The kidney and liver enzymes increased with increasing doses whereas the histopathological damage after long administration on these organs was mild.

In order to narrow down on which secondary metabolite is responsible for the potency of ethyl acetate fraction, it was subjected to VLC using solvents of varying polarity. This resulted in 15 pooled subfractions. Bioassay guided phytochemical screening performed on each of the pool showed that subfraction D1 at 100mg/Kg has no activity, as well as 5% tween 20 used as control. At the same dose, out of the three mice used, for subfractions, D2, D5 and D1O, two mice survived each subfraction for the ten days treatment. For D6 and D13 subfraction, only one mouse survived each administration, whereas in D7 all the three mice survived and the result was similar to the activity of the standard drug - Artemether/lumefantrin.

HPLC-DAD assay carried out on the 19 subfractions identified the following secondary metabolites:

- **Citreodrimene F**

Citreodrimenes (A-F) are sesquiterpene lactones, and this group of compounds occurs most widely in many plant families, primarily the Compositae (Asteraceae). Citreodrimenes are known for their various biological activities, including cytotoxicity to tumor cells (Beekman *et al*., 1997). Citreodrimenes (A-F) have also been isolated from endophytic fungi (*Penicillium citreonigrum*) associated with the Indonesian plant *Pseudocertina purpurea* (Rusman, 2006).

Sesquiterpene lactones (SLs) are probably the largest class of secondary metabolites in plants, with over 5000 structures reported to date (Ivanescu *et al*., 2015). Although SLs are present in approximately 16 plant families, they are prevalent in *Asteraceae* family where they can be found in almost all genera, notably in *Artemisia*, *Arnica*, *Ambrosia*, *Helenium*, *Tanacetum*, and *Vernonia* (Amorim *et al*., 2013; Kreuger *et al*., 2012; Ivanescu *et al*., 2015).

Sesquiterpene lactones are generally known to possess antiplasmodial/antimalarial properties (Lang *et al*., 2002; Chea *et al*., 2006). Several types of sesquiterpene lactones exist including the eudesmanolide, and guaianolide, germacranolides, and pseudoguaianolides types (Lang *et al*., 2002; Lang *et al.*, 2000, Lang *et al.*, 2001; Ivanescu *et al*., 2015; Sülsen *et al*., 2011). This important group of compounds has been identified or isolated from several plants including *Eupatorium semialatum* (Lang *et al*., 2002), *Vernonia cinerea* (Chea *et al*., 2006), *Ambrosia tenuifolia* (Sülsen *et al*., 2011), *Artemisia annua* (Ivanescu *et al*., 2015), etc.

Since the discovery of the use artemisinin against chloroquine-resistant malaria, attention has been turned to other sesquiterpene lactones as a potential source of antimalarial drugs (Sülsen *et al*., 2011). Artemisinin and its analogues show marked activity against *Plasmodium*

species *in vivo* and *in vitro*. It is effective even against multidrug resistant strains of the malaria parasite and in cases of cerebral malaria. Nowadays, artemisinin and its derivatives are recommended by the World Health Organisation to be used as first choice therapy in the treatment of malaria as part of ACT (artemisinin combination therapy) (Ivanescu *et al*., 2015). Although artemisinin, a sesquiterpene lactone, is the most powerful drug against chloroquine-resistant malaria, resistance to this drug might soon appear (Sülsen *et al*., 2011). It is therefore important to find other potent antimalarial compounds.

Other sesquiterpene lactones, ridentin and hanphyllin, isolated from *Artemisia* species also showed antimalarial properties. Ridentin and hanphyllin had inhibitory concentrations (IC50) of 5.4 and 2.3 μg/mL against *Plasmodium falciparum*, respectively. The antimalarial activity may be attributed to the exomethylene group of the lactone function (Ortet *et al*., 2008).

- **Palitantin**

  Palitantin was detected as the most abundant compound in the plant since it was present in most fractions and subfractions of the plant extract. The compound is a known antiprotozoal agent with antileishmanial activity (Fuska *et al*., 1970). Palitantin is a biologically active cyclohexane compound which was originally isolated from the mould - *Penicillium palitans* (Birkinshaw and Raistrick, 1936). The compound is also reported to possess antimicrobial and anti-HIV activities (Chaplen and Thomas, 1960; Hong *et al.* 2007; Torres, 2013).

- **Cytosporin D**

  Cytosporins are a type of hexahydrobenzopyran derivates. Cytosporin D has been reported as a natural product found in *Pestalotiopsis* species and *Eutypella scoparia* (Ciavatta *et al*., 2008; Ding *et al*., 2011). Cytosporin D is assumed to be the precussor of pestaquinols A and

B which contain a novel nonacyclic skeleton and showed cytotoxicity against HeLa cells (Vannada *et al*., 2013).

- **Septicine**

  Septicine is an alkaloid compound present in plants. This compound has been shown to possess anti-inflammatory properties (Geun-Mook and Jin-Kyung, 2011).

- **Aureonitol**

  Aureonitol is a derivative of tetrahydrofuran (THF), and this group of compounds has been shown to possess antiviral activity (Yedidi *et al*., 2014; Zhang *et al*., 2013). Aureonitol has demonstrated excellent antiviral activity against influenza virus (Sacramento *et al*., 2015).

- **Cerebroside**

  Cerebroside is the common name for a group of glycosphingolipids called monoglycosylceramides. They contain a carbohydrate linked by a glycosidic linkage at the 1-hydroxyl position of the long-chain base. Cerebrosides exist in plants as glucocerebrosides (glucosylceramides). Cerebrosides are reported to possess antimicrobial and anti-proliferation properties (Lynch and Dunn, 2004; Poumale *et al.*, 2011; Mbosso *et al.*, 2012).

- **Shanzhiside methyl ester**

  Shanzhiside methyl ester has been reported to possess anti-HCV entry and anti-infective properties (Zhang *et al*., 2009).

The biological activities of the detected compounds are presented in Table 16.

Table 16: Reported bioactivities of detected compounds

Compounds detected	Bioactivity	References
Palitantin	Antiprotozoal, Antimicrobial, HIV-1 integrase inhibition	Chaplen and Thomas, 1960; Fuska *et al*., 1970; Hong *et al.* 2007; Torres, 2013.
Aureonitol	Antiviral	Sacramento *et al*., 2015
Cytosporin D	Cytotoxicity	Vannada *et al*., 2013
Septicine	Anti-inflammatory, antioxidant and antitumour	Geun-Mook and Jin-Kyung, 2011; Park and Kim, 2011
Citreodrimene F	Cytotoxicity, Antimalarial	Beekman *et al*., 1997; Lang *et al*., 2002; Ivanescu *et al*., 2015; Sülsen *et al*., 2011
Cerebroside	Anti-proliferation	Lynch and Dunn, 2004
Shanzhiside methyl ester	anti-HCV entry and anti-infective	Zhang *et al*., 2009

5.2 CONCLUSION

The ultimate aim of bioactive metabolite screening for novel compounds is to isolate compounds which are safe and efficacious for human use or for further investigation or as lead compound. Due to the development of resistance of the malarial parasite to existing compounds and the toxicity of drugs used in the treatment of malaria, the search for newer and safer drug molecules are expedient. The discovery of novel therapeutic molecules from plants is an important alternative to overcome the increasing levels of antimalarial drug resistance and the declining number of potent, safe and nontoxic drugs available against the disease.

In this study, nine plants were evaluated for their malarial activity and *B. pubescens* showed the best activity. Methanol crude extract of *B. pubescens* was fractionated into *n*-hexane, ethyl acetate and butanol fractions. The ethyl acetate fraction was the most effective against malarial parasite. Using VLC and Sephadex seperation techniques, some sub- fractions were obtained which where all subjected to antimalarial screening. HPLC-DAD analysis of the sub-fractions revealed the presence of citreodimene F, a sesquiterpene lactone generally known for its antimalarial properties (Beekman *et al*., 1997; Lang *et al*., 2002; Ivanescu *et al*., 2015; Sülsen *et al*., 2011.). This compound may therefore be responsible for the antimalarial property of the plant. Also, the abundance of palitantin, an antiprotozoal agent with antileishmania properties, was revealved by the HPLC-DAD analysis. This compound may confer an antileishmanial activity on the plant. The plant studied also contained other constituents such as aureonitol, cytosporin D, septicine, cerebroside and shanzhiside methyl ester with broad range of pharmacological activities such as antimicrobial, anti-inflammatory, anti-viral and cytotoxic activities. Toxicological studies on the plant showed *B. pubescens* to quite safe on the major organs studied.

This study is part of efforts and resources geared towards the malaria control in Nigeria, where the burden of malaria is greatest in Africa due to the large population. It is hoped that the information and data present herein will be useful in rolling back this disease.

5.3 RECOMMENDATIONS

There are several different approaches to antimalarial drug discovery, and in all of these approaches, it is necessary to take into account specific concerns, including the need to limit the cost of drug discovery and the cost of the drug itself. Several important ongoing efforts include the following: optimization of therapy with available drugs, including the use of combined therapy; development of analogues of existing agents; discovery of natural antimalarial products; investigation of compounds that were originally developed to treat other diseases; evaluation of drug resistance reversers; and chemotherapeutic exploitation of novel molecular targets (Rosenthal 2001, 2003).

It is obvious that the prime consideration in antimalarial drug development is economic in nature. Since the drugs are likely to be used in endemic areas which exist in underdeveloped countries with high poverty levels, the first factor to be considered in antimalarial drug discovery is that the drugs must be very inexpensive. Secondly, since markets for malaria are primarily in poor countries, marketing opportunities are generally limited; therefore, investment in antimalarial drug discovery and development has been insubstantial and highly dependent on support from outside of the large pharmaceutical companies. Such support includes grants that are issued to academic and industry groups by research agencies and new public-private partnerships; however, this imbalance remains large (Rosenthal 2003; Oliveira *et al*., 2009).

The validation of traditional plant remedies has limitations, such as the prioritization of plant species for research, a lack of information on the ethnobotany of these plants (location and abundance, parts used, form of use, duration of treatment), and the definition of dosages due to variations in the concentrations of active ingredients in a plant species (Willcox and Bodeker 2004, Bourdy *et al*. 2007). It should be stressed that a basic requirement for the validation of a medicinal plant is the standardization of the extracts that are to be evaluated. Standardization includes identification and quantification of chemical and/or biological markers to ensure the development of efficient and safe phytomedicines in a short period of time and at a low cost. It is well known that both the qualitative and quantitative contents of secondary metabolites in a plant are susceptible to marked variations; these contents are influenced by intrinsic factors (ontogeny and phenology), abiotic factors (light, moisture, nutrient availability), and biotic factors (different physiological and growth stages. As a result, standardization is obligatory (Oliveira *et al*., 2009; Harborne 2001).

Since palitantin, an antiprotozoal agent with antileishmania properties was revealved by HPLC-DAD analysis to be in high abundance in *B. pubescens*, it can be assumed that this compound may confer antileishmanial activity on the plant. It is therefore recommended that research be carried out to evaluate the antileishmanial activity of the plant.

5.4 CONTRIBUTION TO KNOWLEDGE

This study reports the antimalarial screening of nine Nigerian plants used ethnomedicinally for the treatment of malaria. The results of this study provide a scientific evaluation and validation of the traditional application of these plants in malarial therapy. It was observed that of all the plants, *B. pubescens* was the most effective. Also, together with the confirmation of the

antimalarial activity of the plant, results of the toxicological studies confirm the safety of the plant.

Chemical investigations of the plant revealed the presence of important biologically active compounds including citreodimene F, palitantin, aureonitol, cytosporin D, septicine, cerebroside, shanzhiside methyl ester. This is the first report of the detection of these compounds from *B. pubescens.*

The findings of this study are the first of its kind and it is hoped that the information and data present herein will be useful in rolling back of malaria.

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APPENDICES

Table A: Result of percentage cure of the different plants

	Dose mg/Kg	Mean Basal count	Mean final count	Cure (%)
Anthocleista djalonensis	50	24.00 ± 0.71	13.40 ± 0.51	44.17
	100	26.00 ± 0.71	12.60 ± 0.93	51.54
	200	26.00 ± 0.71	11.60 ± 0.51	55.38
Baphia pubescens	50	24.00 ± 0.71	1.60 ± 0.40	93.33
	100	27.00 ± 0.71	0.80 ± 0.37	97.04
	200	26.00 ± 0.71	1.00 ±0.32	96.15
Buccholzia coriaceae	50	25.00 ± 0.71	9.40 ± 0.40	62.40
	100	33.00 ±0.71	7.20 ± 0.37	78.18
	200	30.20 ± 0.80	5.40 ± 0.51	82.12
Synclesia scabrida	50	21.00 ± 0.71	10.40 ± 0.51	50.48
	100	27.20 ± 0.58	6.40 ± 0.51	76.47
	200	28.00 ± 0.71	3.40 ± 0.25	87.86
Rauwalfia vomitoria	50	23.80 ± 0.86	3.80 ± 0.37	84.03
	100	30.60 ± 0.68	3.20 ± 0.58	89.54
	200	30.00 ± 0.71	0.80 ± 0.37	97.33
Moringeroleifera	50	23.00 ± 0.71	10.60 ± 0.51	53.91
	100	26.40 ± 0.51	10.00 ± 0.71	62.12
	200	30.20 ±0.86	7.20 ± 0.58	76.16
Kigeriaafricana	50	26.00 ± 0.71	5.80 ± 0.37	77.69
	100	29.20 ± 0.86	2.80 ± 0.37	90.41
	200	30.20 ± 0.86	0.80 ± 0.37	97.35
Morinda lucida	50	23.80 ± 0.86	6.20 ± 0.37	73.95
	100	28.20 ± 0.86	5.40 ± 0.51	80.85
	200	25.60 ± 0.51	3.40 ± 0.51	86.72
Nauclea latifolia	50	27.20 ± 0.58	12.60 ± 0.51	53.68
	100	30.00 ± 0.71	12.00 ± 0.71	60.00
	200	31.20 ± 0.86	10.00 ± 0.71	67.95
Quinine	50	21.20 ± 0.58	0.40 ± 0.25	98.11
Tween-80,		18.00 ± 0.71	20.00 ± 0.71	0

Table B: Results of percentage cure of ethyl acetate fractions

	Dose	Mean Basal count	Mean final count	Cure (%)
Tween-80,		18.00 ± 0.71	20.00 ± 0.71	0
Quinine	50 mg/Kg	21.20 ± 0.58	0.40 ± 0.25	98.11
Ethyl acetate fraction	100 mg/Kg	28.00 ± 1.22	1.30 ± 0.54	95.36
n-hexane fraction	100 mg/Kg	26.80 ± 1.39	6.00 ± 0.71	74.29
Butanol fraction	100 mg/Kg	25.20 ± 1.46	2.40 ±0.51	81.43
Methanol fraction	100 mg/Kg	24.20 ± 1.80	2.40 ± 0.51	77.86

Table C: Result of curative test (*In vivo* Antimalarial Activities of sub-fractions o*f Baphia pubescens* Ethyl Acetate Fraction)

Sample code	Animal No.	Weight (grams)	Parasitaemia (Day 3); Before Treatment.	Parasitaemia (Day 8); After Treatment.	Fraction Alive after Day 13
D1	**1**	21	60	-	0/3
	2	20	63	68	
	3	19	68	-	
D2	**1**	18	70	63	2/3
	2	20	62	38	
	3	21	54	-	
D5	**1**	22	65	11	2/3
	2	22	59	34	
	3	20	64	43	
D6	**1**	24	61	-	1/3
	2	18	67	54	
	3	19	61	48	
D7	**1**	19	59	33	3/3
	2	23	63	28	
	3	22	72	32	
D10	**1**	19	74	54	2/3
	2	20	66	21	
	3	21	68	18	
D13	**1**	21	75	38	1/3
	2	22	63	42	
	3	19	78	53	
Artemether/lumefantrin	**1**	22	64	9	3/3
	2	18	63	14	
	3	19	72	29	
5% Tween 20	**1**	23	74	-	0/3
	2	25	68	-	
	3	19	51	-	

Table D: Effect of ethyl acetate fraction of *B. pubescens* on liver enzymes (ALT, AST, and ALP) after 16 weeks of administration

		MEAN	±SEM	P-VALUE
Alanine Transaminase	Group 1	41.25	±2.92	
	Group 2	92.50	±3.22	0.000**
	Group 3	119.00	±1.95	0.000**
	Group 4	121.00	±2.10	0.000**
Aspartate Transaminase	Group 1	28.00	±1.47	
	Group 2	79.50	±6.60	0.002*
	Group 3	135.00	±5.40	0.000**
	Group 4	233.75	±11.43	0.000**
Alkaline Phosphatase	Group 1	54.25	±1.79	
	Group 2	95.00	±9.12	0.017*
	Group 3	151.50	±6.30	0.000**
	Group 4	242.75	±10.60	0.000**

Table E: Effect of ethyl acetate fraction of *B. pubescens* on kidney enzymes (creatinine and urea) after 16 weeks of administration

		MEAN	±SEM	P-VALUE
Urea (mmol/L)	Group 1	44.87	±1.73	
	Group 2	82.25	±5.85	0.014*
	Group 3	132.50	±3.22	0.000**
	Group 4	215.00	±1.90	0.000**
Creatinine (umol/L)	Group 1	54.00	±3.18	
	Group 2	100.00	±7.35	0.012*
	Group 3	138.75	±5.54	0.000**
	Group 4	228.75	±13.28	0.000**

Table F: Effect of ethyl acetate fraction of *B. pubescens* on red blood cell, hemoglobin and pack cell volume after 16 weeks of administration

		MEAN	±SEM	P-VALUE
Red Blood Cell(X 10^6/µL)	Group 1	4.74	±0.09	
	Group 2	5.05	±0.11	0.222
	Group 3	5.00	±0.04	0.436
	Group 4	6.75	±0.10	0.000*
Hemoglobin (g/dL)	Group 1	11.00	±0.40	
	Group 2	12.00	±0.40	1.000
	Group 3	13.50	±0.64	0.040*
	Group 4	15.50	±0.65	0.000**
Pack Cell Volume (%)	Group 1	41.00	±0.91	
	Group 2	42.25	±1.37	1.000
	Group 3	44.25	±1.31	0.331
	Group 4	47.75	±0.47	0.005*

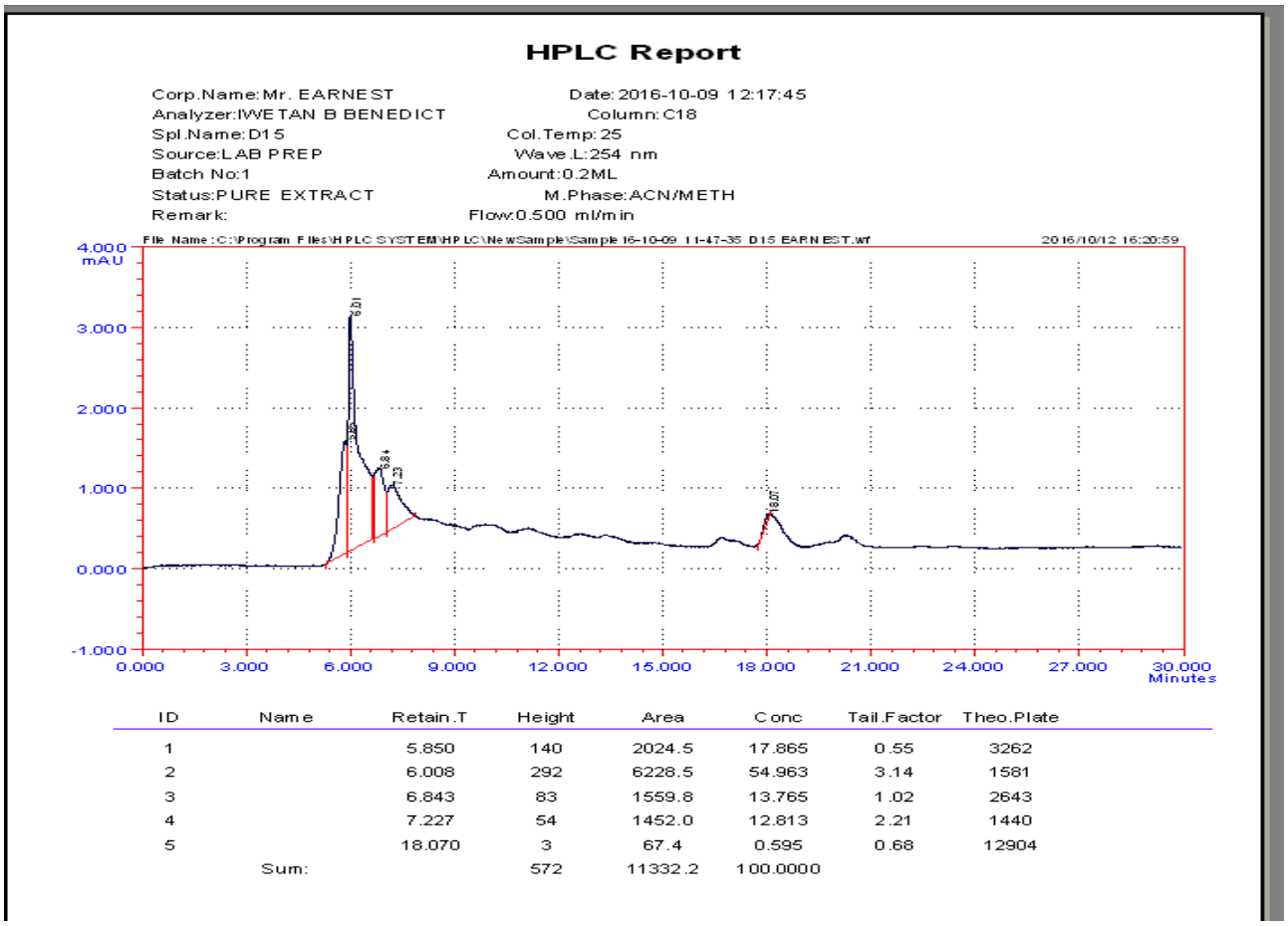
Table G: Effect of ethyl acetate fraction of *B. pubescens* on white blood cells and platelets after 16 weeks of administration

		MEAN	±SEM	P-VALUE
White Blood Cell(X 10^9/L)	Group 1	6.45	±0.15	
	Group 2	7.02	±0.07	0.187
	Group 3	7.22	±0.10	0.039*
	Group 4	7.77	±0.26	0.001**
Platelet (X 10^9/L)	Group 1	236.25	±2.39	
	Group 2	257.50	±3.22	0.024*
	Group 3	274.25	±4.97	0.000**
	Group 4	286.25	±5.54	0.000**

Table H: Effect of ethyl cetate fraction of *B. pubescens* on body weight after 16 weeks of administration

Body weight (g)		MEAN	±SEM	P-VALUE
Group 1	Initial	145.00	±2.88	
	Final	195.00	±13.22	0.027*
Group 2	Intial	170.00	±4.08	
	Final	227.50	±11.08	0.011*
Group 3	Initial	165.00	±6.45	
	Final	212.50	±6.29	0.002**
Group 4	Initial	177.50	±4.78	
	Final	235.00	±6.45	0.003*

HPLC REPORT OF VLC FRACTIONS OF *B. PUBESCENS*



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.850	140	2024.5	17.865	0.55	3262
2		6.008	292	6228.5	54.963	3.14	1581
3		6.843	83	1559.8	13.765	1.02	2643
4		7.227	54	1452.0	12.813	2.21	1440
5		18.070	3	67.4	0.595	0.68	12904
	Sum:		572	11332.2	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B.BENEDICT
Spl.Name:DI
Source:LAB PREP
Batch No:N HEX/ETHYL(5:5)
Status:PURE EXTRACT
Remark:

Date:2016-10-09 13:25:21
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Col.Temp:25
Wave.L:254 nm
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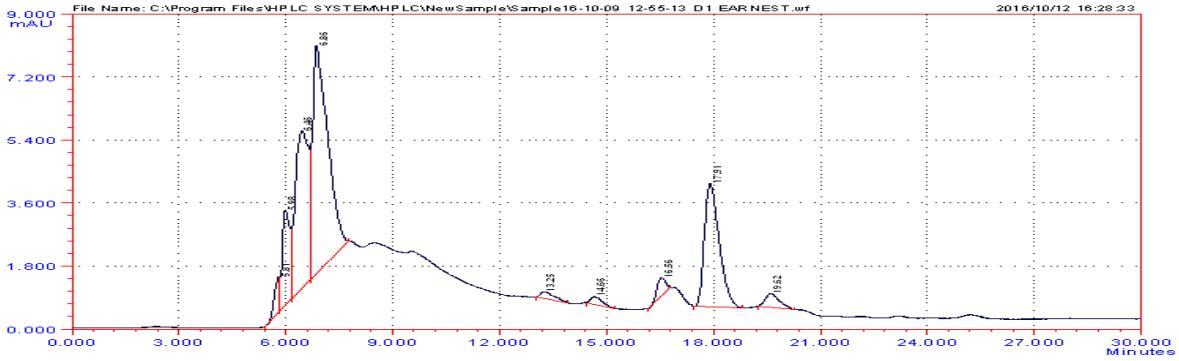
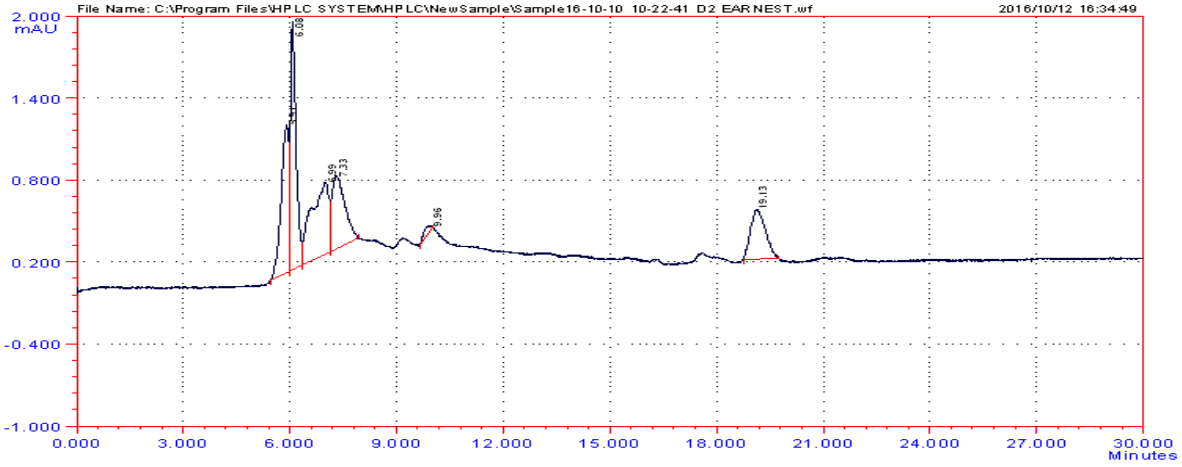
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.808	100	1007.2	2.055	0.56	6628
2		5.983	271	4124.1	8.416	1.12	3081
3		6.460	448	11689.0	23.854	0.90	1222
4		6.860	648	19027.3	38.829	3.18	1088
5		13.255	20	521.0	1.063	1.50	5159
6		14.657	22	493.5	1.007	1.21	8509
7		16.560	52	882.6	1.801	1.14	18972
8		17.912	352	10125.1	20.663	1.36	7728
9		19.623	40	1132.6	2.311	1.20	9573
	Sum:		1953	49002.4	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Date:2016-10-10 10:53:14
Analyzer:IWETAN B BENEDICT
Column:C18
Spl.Name:D2
Col.Temp:25
Source:LAB PREP
Wave.L:254 nm
Batch No:DCM/METH(2:8)
Amount:0.2ML
Status:PURE EXTRACT
M.Phase:ACN/METH
Remark:
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.907	107	1592.6	20.323	0.61	3139
2		6.075	181	1953.3	24.926	1.95	6316
3		6.993	52	1819.8	23.222	0.61	796
4		7.335	53	1330.0	16.971	1.98	1703
5		9.963	6	80.3	1.024	0.70	11054
6		19.128	36	1060.5	13.533	1.30	8404
	Sum:		435	7836.4	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D3
Source:LAB PREP
Batch No:DCM/METH(5:5)
Status:PURE EXTRACT
Remark:

Date:2016-10-10 12:21:29
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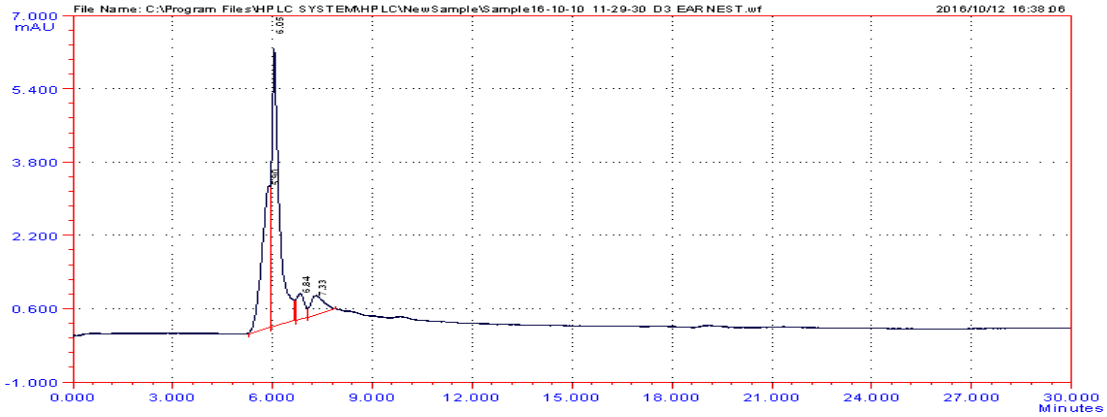
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.900	310	5138.5	30.981	0.53	2525
2		6.050	605	9393.8	56.636	3.05	3026
3		6.845	56	911.2	5.494	1.13	3527
4		7.330	41	1142.7	6.889	1.45	1379
	Sum:		1012	16586.1	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENDICT
Spl.Name:D4
Source:LABPREP
Batch No:DCM/METH(10;0)
Status:PURE EXTRACT
Remark:

Date:2016-10-10 13:01:29
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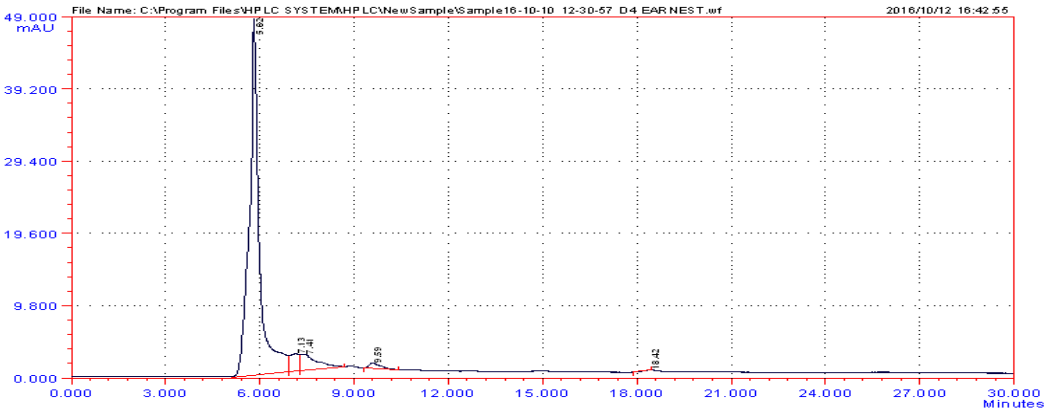
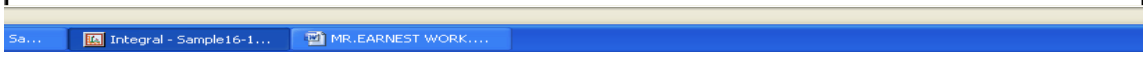
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.817	4840	106984.8	88.129	1.54	1380
2		7.135	240	4612.6	3.800	0.89	2747
3		7.410	224	7985.9	6.578	5.61	861
4		9.588	68	1764.8	1.454	1.37	2720
5		18.420	3	47.0	0.039	0.71	27532
	Sum:		5375	121395.1	100.0000		



HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D5
Source:LAB PREP
Batch No:NHEX/ETHYL(450/50)
Status:PURE EXTRACT
Remark:

Date:2016-10-11 10:20:51
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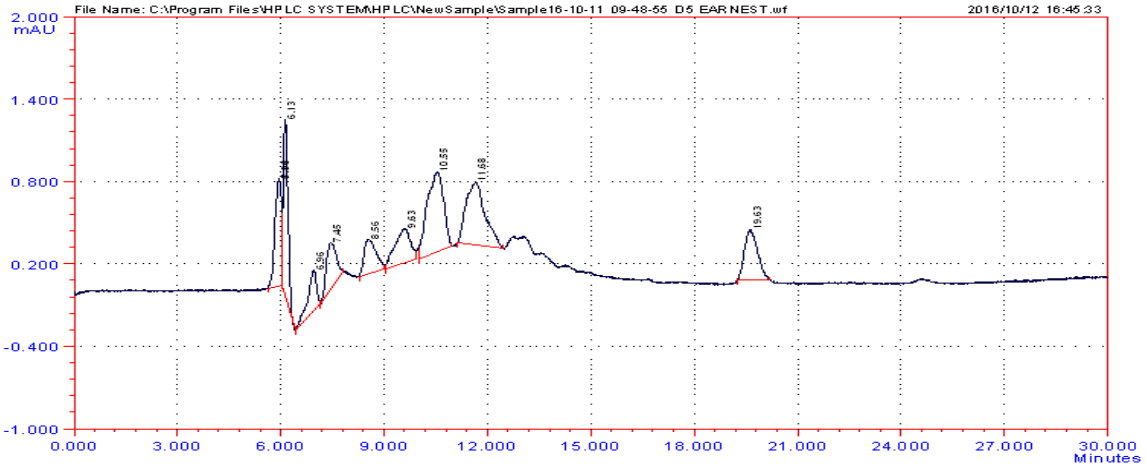
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.958	77	1021.9	10.827	0.66	4017
2		6.133	128	1109.8	11.758	1.44	9973
3		6.960	30	529.5	5.610	0.83	3099
4		7.452	33	662.4	7.018	1.41	2747
5		8.562	25	610.1	6.464	1.40	2453
6		9.630	24	783.0	8.295	0.75	1737
7		10.548	58	1961.2	20.778	0.88	1940
8		11.675	46	1773.6	18.791	1.13	1827
9		19.630	36	987.2	10.459	1.24	10213
	Sum:		457	9438.6	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D6
Source:LAB PREP
Batch No:NHEX/ETHYL(6:4)
Status:PURE EXTRACT
Remark:

Date:2016-10-11 11:54:22
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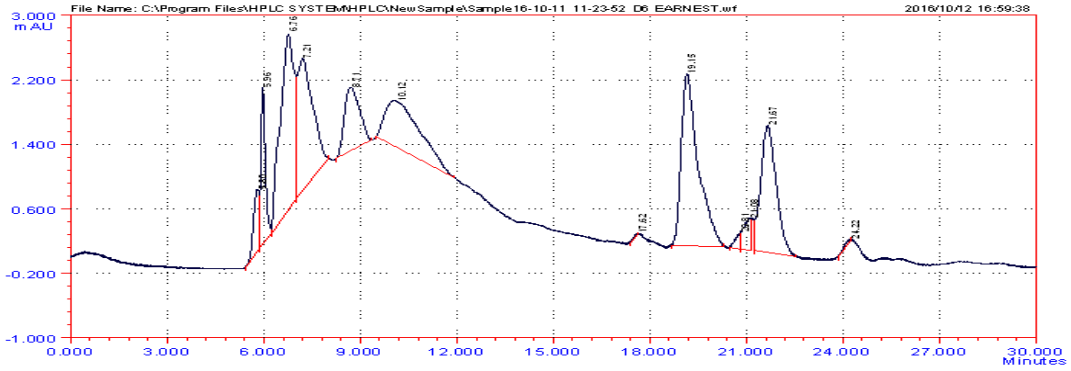
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.800	76	889.2	2.496	0.64	4898
2		5.958	195	1893.4	5.315	1.79	7505
3		6.760	218	6719.5	18.863	0.78	959
4		7.210	164	5157.7	14.479	2.62	1048
5		8.713	79	2593.4	7.280	1.37	1404
6		10.123	56	4447.6	12.485	1.36	324
7		17.620	2	25.6	0.072	0.62	37747
8		19.155	213	7749.6	21.755	1.64	5524
9		20.808	20	215.3	0.604	0.53	74494
10		21.083	40	577.0	1.620	0.60	42575
11		21.668	157	5311.5	14.911	1.32	8176
12		24.222	2	42.5	0.119	0.65	25923

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D7
Source:LAB PREP
Batch No:NHEX/ETHYL(7:3)
Status:PURE EXTRACT
Remark:

Date:2016-10-11 13:05:06
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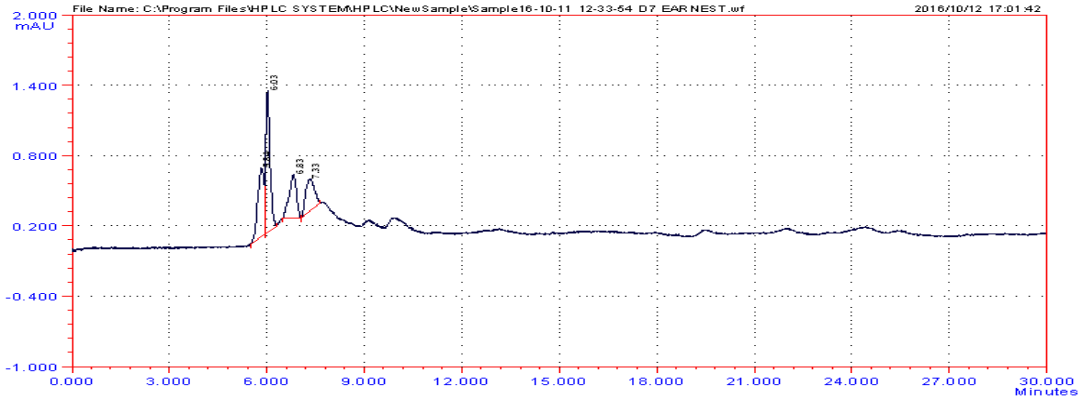
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min

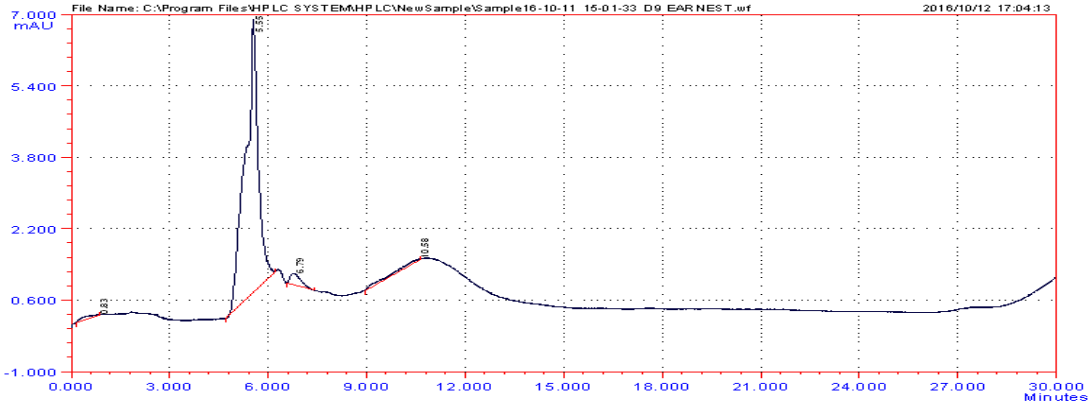


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.842	58	761.0	25.819	0.70	3951
2		6.025	120	1080.7	36.668	1.91	8920
3		6.827	37	581.7	19.737	0.81	3757
4		7.335	27	523.9	17.776	1.18	2848
	Sum:		242	2947.4	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D9
Source:LAB PREP
Batch No:dcm/meth(8:2)
Status:PURE EXTRACT
Remark:

Date:2016-10-11 15:31:47
Column:C18
Col.Temp:25
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min

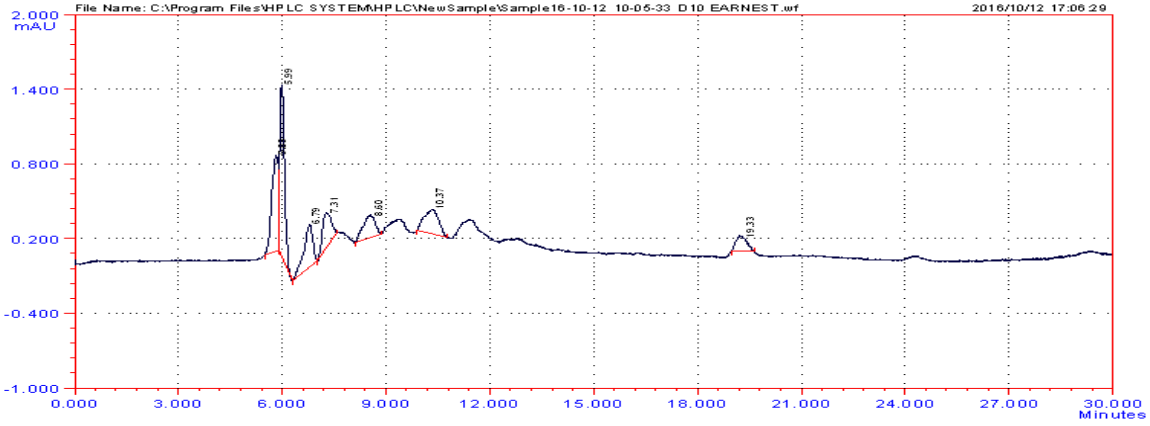


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.833	1	183.3	1.031	0.02	0
2		5.548	611	16688.5	93.862	1.05	822
3		6.792	26	595.0	3.347	1.23	1755
4		10.582	1	312.9	1.760	0.02	23
	Sum:		639	17779.8	100.0000		

HPLC Report

Corp.Name:Mr.EARNEST
Analyzer:IWETAN B.BENEDICT
Spl.Name:D10
Source:LAB PREP
Batch No:NHEX/ETHYL(8:2)
Status:PURE EXTRACT
Remark:

Date:2016-10-12 10:35:56
Column:C18
Col.Temp:25
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min

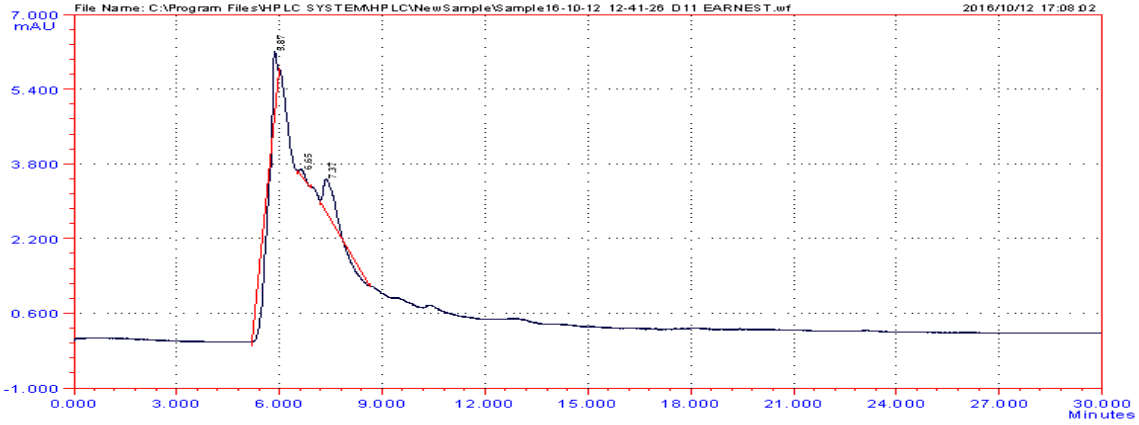


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.833	77	992.7	20.965	0.61	4081
2		5.992	138	1308.4	27.633	1.47	7960
3		6.793	35	648.0	13.686	0.84	2683
4		7.312	27	501.2	10.585	1.17	3092
5		8.597	18	442.4	9.344	0.84	2438
6		10.367	19	571.6	12.072	0.84	2367
7		19.332	12	270.6	5.715	0.93	14649
	Sum:		326	4734.8	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B. BENEDICT
Spl.Name:D11
Source:LAB PREP
Batch No:NHEX/ETHYL(3:7)
Status:PURE EXTRACT
Remark:

Date:2016-10-12 13:11:42
Column:C18
Col.Temp:25
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min

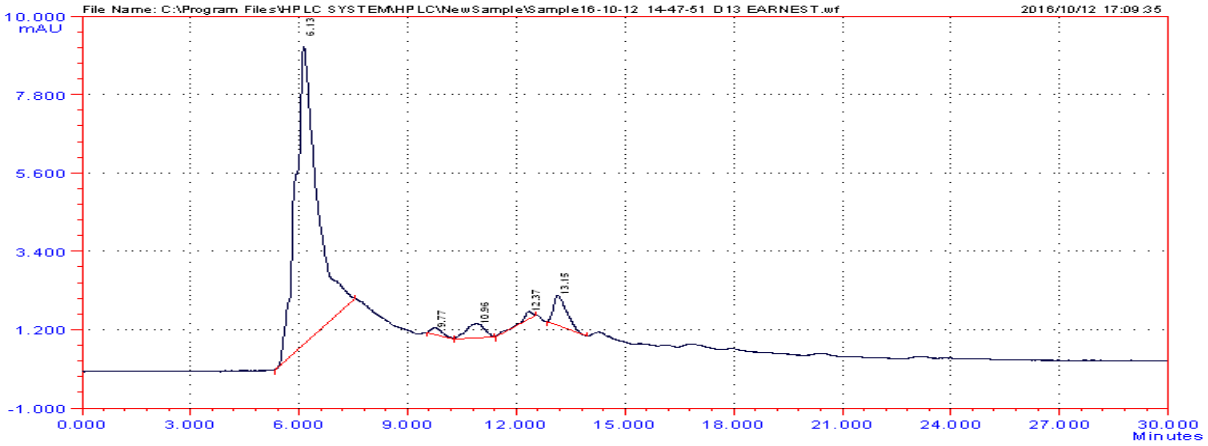


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.867	134	1179.1	39.596	1.37	8859
2		6.650	10	126.9	4.262	0.93	5471
3		7.369	71	1671.9	56.142	1.30	1952
	Sum:		215	2977.9	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D13
Source:LAB PREP
Batch No:NHEX/ETHYL(4:6)
Status:PURE EXTRACT
Remark:

Date:2016-10-12 15:22:17
Column:C18
Col.Temp:25
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min

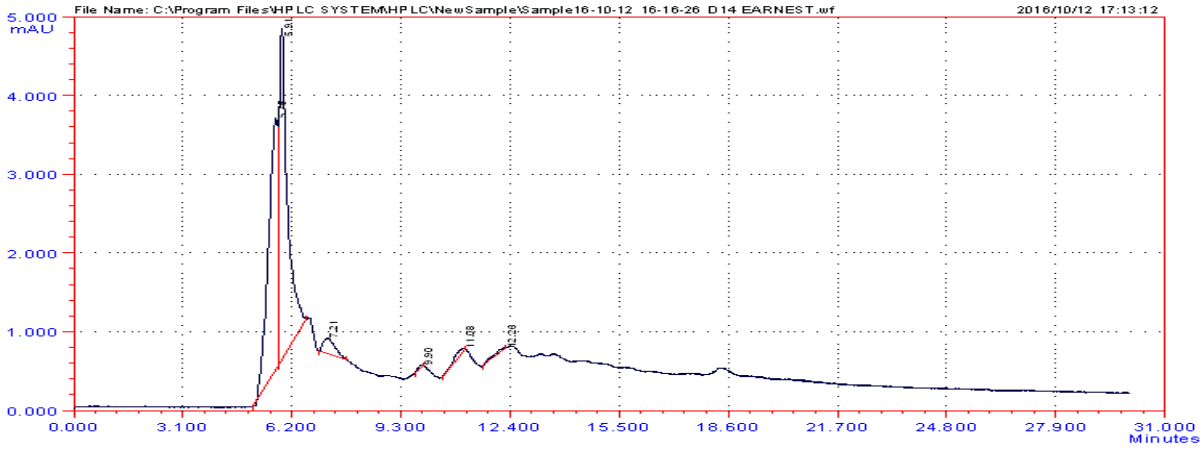


ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		6.133	832	34014.2	89.128	1.71	449
2		9.765	17	354.7	0.929	1.06	4366
3		10.958	39	1335.7	3.500	0.88	2041
4		12.368	19	303.7	0.796	1.12	11932
5		13.153	84	2155.2	5.647	1.20	5238
	Sum:		991	38163.4	100.0000		

HPLC Report

Corp.Name:Mr. EARNEST
Analyzer:IWETAN B BENEDICT
Spl.Name:D14
Source:LAB PREP
Batch No:NHEX/ ETHYL(0:10)
Status:PURE EXTRACT
Remark:

Date:2016-10-12 16:47:39
Column:C18
Col.Temp:25
Wave.L:254 nm
Amount:0.2ML
M.Phase:ACN/METH
Flow:0.500 ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		5.747	319	5791.9	43.188	0.58	1997
2		5.914	420	6919.3	51.594	3.97	2568
3		7.209	20	472.8	3.526	1.25	1853
4		9.903	2	35.6	0.265	0.74	6180
5		11.080	3	96.6	0.721	0.66	2358
6		12.260	2	94.7	0.706	0.02	1337
	Sum:		766	13410.9	100.0000		