IN VITRO ANTIOXIDANT AND ANTIMALARIAL PROPERTIES OF THE PLANTS *Chasmanthera dependens* (Hochst) and *Dictyandra arborescens* (Welw.)

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CERTICATION

We hereby certify that this thesis **"*In Vitro* Antioxidant and Antimalarial Properties of plants *Chasmanthera dependens* and *Dictyandra arborescens*"** was carried out by UCHE EMMANUELA ENENEBEAKU with Reg. No. 20154949498, in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biotechnology, in the Department of Biotechnology, Federal University of Technology Owerri.

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DEDICATION

This work is dedicated to my husband Prof. Kenechukwu Conrad Enenebeaku, my children Kenechukwu (Jnr), Chikamso and Chukwuagoziem, to my parents, Sir. & Lady B.C. Odionye and my siblings for their encouragement and support throughout the production of this work. Also, to the memory of my supervisor Late Prof. N.C. Ukwandu who designed and completed the supervision of this work before his death.

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ABSTRACT

Crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* suggested from their ethno-medicinal uses to possess antimalarial activities were evaluated for phytochemical, *in vitro* antioxidant, toxicity, and antimalarial activities. Phytochemical analyses were carried out using standard qualitative and quantitative analytical methods. *In vitro* antioxidant potentials of the extracts were determined by their abilities to scavenge 2, 2-diphenyl-1- picrylhydrazyl radical, nitric oxide radical and hydrogen peroxide. The antioxidant potentials were further determined by the ferric reducing antioxidant power and total antioxidant capacity using standard methods. Acute toxicity was carried out using modified Lorke's method, sub-acute toxicity of the extracts were also determined using adult male albino rats. Antimalarial activities of the extracts were determined using male Swiss albino mice infected with *Plasmodium berghei*. Methanolic extracts of *D.arborescens* roots which showed the highest antimalarial activity was subjected to separation by silica gel column chromatography while bioassay was carried out by testing the antimalarial activities of the fractions on male Swiss albino mice. Hexane fraction of the extract showed the highest antimalarial activity and was sub-fractionated to obtain two purer eluates coded $\mathbf{E_A}$ and $\mathbf{E_B}$ which were characterized using Gas Chromatography – Flame Ionization Detector (GC-FID), Fourier Transform Infrared (FTIR) spectroscopy, Gas Chromatography-Mass Spectrometry (GC-MS) and Molecular docking analyses. Phytochemical screening of the extracts revealed the presence of tannins, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, phenols, flavonoids, oxalate and phytate in varying amounts. The phytochemicals were more in the methanolic extracts than in the aqueous extracts. Scavenging of DPPH, nitric oxide, H_2O_2 radicals, reducing power ability and total antioxidant activity were concentration-dependent. Activities of the extracts for DPPH, nitric oxide and H_2O_2 differed significantly ($p<0.05$) from the standard antioxidant. For ferric reducing antioxidant power, methanolic extract of *D.arborescens* roots and methanolic extract of *C. dependens* roots showed the best reducing ability. Methanolic extract of *D. arborescens* root showed the highest (2352.94 ±164.87mg AA/g extract) total antioxidant capacity. Extracts of the leaves, stem and roots of *D. arborescens* did not show any mice toxicity or mortality even at 5000 mg/kg body weight but aqueous and methanolic extracts of *C. dependens* stem recorded death at 2900 mg/kg b.wt and 5000 mg/kg b.wt. Sub-acute oral toxicity test of the extracts revealed significant increase ($p <0.05$) on haematological parameters (PCV, Hb, RBCs, WBCs, MCH, Neutrophils, Lymphocytes and MCHC) at 200 mg/kg^{-1} body weight. For biochemical parameters (ALT, AST, ALP, total protein, and albumin), extracts did not significantly differ ($p<0.05$) from the standard and normal control. All the extracts showed significant ($p<0.05$) antimalarial activities compared to the untreated control. These activities however, differed ($p<0.05$) from the standard antimalarial drug (artesunate). Methanolic extract of *D.arborescens* root showed the highest antimalarial activity by reducing percentage parasitemia from 75.2% on day 3 to 16.8% on day 14 after treatment. Column chromatographic separation of this extract gave five fractions at different solvent mixtures. Bioassay revealed that hexane soluble fraction recorded the highest antimalarial activity, and sub-fractionation of this fraction yielded two purer eluates -$\mathbf{E_A}$ and $\mathbf{E_B}$**.** Phytochemical analysis with Gas chromatography- Flame ionization detector (GC-FID) revealed various phytochemicals with known antimalarial and antioxidant activities. FTIR spectroscopy revealed the presence of functional groups such as alkanes ($-CH_2-$), α-halogeno carboxylic acids (C=O), primary alcohols (C- OH), halides (C-Cl), non conjugated alkenes (C=C), esters (OC – H), aliphatic esters (R-O-R), etc. These functional groups were confirmed by GC-MS analysis which

recorded various bioactive compounds such as 1,2 dichloro propane, 1-Octadecene, cis- alpha-bisabolene, Dichloroacetic acid tridecyl ester, 9,17 Octadecadienal, (Z)-, cis-13-Octadecenoic acid, Heptadecanoic acid, 16 methyl-, methyl ester, Bis (2-ethylhexyl) phthalate, Bis(3-methylbutan-2-yl) phthalate, Carbonic acid prop-1-ene - 2yl-tetra, 5-Octadecene, (E)-, Hexadecanoic acid methyl ester, 10-Octadecenoic acid methy ester Butyl octadecyl ether methyl tetradecanoate, Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 tetradecamethyl. In molecular docking analysis, these bioactive compounds showed various degrees of binding affinities and molecular interactions with two antimalarial protein targets - lactate dehydrogenase (1OC4) and Plasmepsin II (1SME). Binding affinity of Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate were close to that of the standard antimalarial drug (artesunate) suggesting that these two compounds were responsible for the antimalarial activity exhibited by methanol extract of *D.arborescens* root. ADMET properties of these two compounds equally suggest no harmful or toxic effects when compared to the standard antimalarial drug (artesunate).

Keywords: *Chasmanthera dependens*, *Dictyandra arborescens*, Phytochemicals, *In vitro* antioxidants, Toxicity, Antimalarial, Isolation, Molecular docking

CHAPTER I: INTRODUCTION

1.1 Background of Study

Malaria remains one of the world's most dreaded and life-threatening parasitic diseases (Adefioye Ad-eyeba, Hassan & Oyeniran, 2007; Okonko, Soleye, Amusan, Ogun, Udeze, Nkang, Ejembi & Faleye, 2009; Olasehinde, Ajayi, Taiwo, Adekeye & Adeyeba, 2010; Abah & Temple, 2015). It is a major disease of public health importance and one of the oldest parasitic diseases of man (Kunihya, Samaila, Pukuma & Qadeer, 2016). It is a major threat to public health and economic development in Sub-Saharan Africa. The disease was originally thought to come from filthy environments, hence the name "*mal aria*" meaning bad air (Banek, Lalani, Steadke & Chandramohan, 2013). This belief continued to hold until scientists discovered that the parasite responsible for this disease is transmitted from one person to another through the bite of female *Anopheles* mosquitoes. Various studies reported that the disease causing agent transmitted by these mosquitoes was a unicellular parasite called *Plasmodium* whose infective sporozoite stage is carried in the mosquito's salivary glands (Ani, 2004; Akinleye, 2009; Okonko *et al*., 2009; Olasehinde *et al*., 2010; Unata, Bunza, Ashcroft, Abubakar & Faruk, 2015; Tela, Modibbo, Adamu & Taura 2015; Kunihya, Samaila, Pukuma & Qadeer, 2016; Udoh, Hamidu & Saleh, 2016). There are four species of this disease causing agent in humans namely *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium falciparum*. Most malaria infections in Sub-Saharan Africa are due to *Plasmodium falciparum* (Idowu, Soniran, Ajana & Aworinde, 2009, Udoh *et al*., 2016). This species is equally accountable for most fatal and severe cases of malaria in Africa (Ani, 2004). Malaria is highly prevalent in tropical climates, where rainfall is sufficient for mosquitoes to breed and the temperatures are conducive for both the mosquito and protozoa to

live. These environmental conditions favour the breeding and rapid multiplication of *Anopheles* species resulting in high resistance to pesticides (Murray, Rosenfeld, Lim & Lopez, 2012).

About 40% of the world population is at risk of this life threatening disease because they live in malaria endangered areas (Adebayo, 2011; WHO, 2013). Annually, an estimated three million deaths occur throughout the world as a result of malaria, with Africa constituting more than 90% of the cases and 75% of deaths (Breman, Alilio, & Mills, 2014; WHO, 2016). This development has a huge impact on the mortality and morbidity rate resulting from malarial infection, particularly in Africa where the most dangerous of all the species, *Plasmodium falciparum,* accounts for approximately 90% of deaths. Malaria has been implicated as a major cause of death of about two million children globally per annum (Okafor & Oko-Ose, 2012).

In Nigeria, presence of malaria is horendous (every individual in the population is a possible target) as reported by Garba, Muhammad, Edem, Adeniji, Aghadueki, Kolawole & Adelakun (2014), Okoli and Solomon (2015). Up to 90% of the population lives in endemic areas (FMoH, 2013). The disease is a major cause of childhood and maternal mortality. About 300,000 or more deaths occur annually as a result of malaria and deaths are due to severe and complicated malarial attacks, particularly in rural areas, with children and pregnant women being more at risk (Salako, 2014). Similarly, Federal Ministry of Health (2014) stated that about 95-99% of adult population in Nigeria carries the malaria parasite although less than 30% of this figure displays symptoms of the illness.

Generally, malaria is seen as a disease of poverty and developing countries. Its transmission and prevalence, according to (Murray *et al*., 2012 & World Health Organization, 2013), is still responsible for thousands of deaths per annum, especially in the tropical regions. The disease is

more prevalent in rural areas where poverty, ignorance to good hygienic practices, poor health care facilities and infrastructures, as well as circulation of fake or counterfeit drugs and pharmaceuticals join forces to worsen the predicaments of the less privileged (WHO, 2013). According to Iloh, Orji and Amadi (2013); Nmadu, Peter, Alexander, Koggie & Maikenti, (2015); Bassey and Nwakaku (2017), malaria is holoendemic (persistence of high level of incidence of the disease) in the rural areas and mesoendemic (affecting a moderate proportion of the population) in the urban areas in Nigeria. In endemic areas, it is a major threat to both economic and human capital development (Nwanosike, Ikpeze & Ugbor, 2015; Ukpong, Etim, Ogban & Abua, 2015).

Malaria is accountable for about 60% of out-patient hospital visits and 30% of hospital admissions among children below the age of five years (Nwanosike, Ikpeze & Ugbor, 2015). A report by the Nigerian malaria fact sheet (2011) and World Health Organization (WHO, 2016) stated that approximately 97% of Nigerians live in high malaria transmission areas, while the remaining 3% live in low malaria transmission areas. This makes her the highest bearer of the disease burden in the African region (WHO, 2016). This high prevalence has been attributed to rainfall, numerous stagnant water, warm temperature, type and distribution of vegetation cover, poor sanitary conditions etc which favour the breeding and multiplication of mosquitoes (Ukaegbu, 2014).Yet, Pats and Olson (2006); and Stresman (2010) have projected an increase in this figure as a result of climate change.

The disease is not only detrimental to the health and lives of Africans, and Nigerians in particular, it equally drains the economy. This is due to reduction in manpower as well as cost of treatment and high death rates associated with the disease. This has made malaria a major retarding factor in African development (Ekpenyong & Eyo, 2008). Since many malaria endemic countries are

reffered to as poor, malaria therefore maintains a vicious cycle of disease and poverty in the African region. In 2012, World Health Organization and World Bank described malaria as a heavy burden on Africa, responsible for a loss of about 35 million lives due to disability and premature mortality (WHO, 2012).

As many communities in Africa engage in subsistence agriculture, time lost due to malaria results in less food production and consumption. This is because the season of high malaria transmission (wet season) coincides with that of intense activities in the farm. Giradin, Dao, & Koudou (2014) stated that a short period of illness can therefore have serious adverse consequences on the economy and nutritional status of these farmers. Given that majority of the population are farmers, malaria infection can become a determining factor in choice of crops to be planted. Less labour intensive crops are preffered to more profitable and labour intensive ones. This serves as a coping mechanism to back up labour shortage caused by malarial attacks (Hoek, 2004). Fear of being infected with malaria can also discourage foreign trade and investment. This consequently makes Africa to remain isolated and poor in a rapidly globalizing economy.

Majority of deaths due to malaria occur in sub-saharan Africa (SSA), and most of the victims are children under the age of five years (WHO, 2013). Pregnant women also suffer severe undesirable effects of malaria. It has been implicated as the major cause of stillbirths, infant mortality, abortion and low birth weight, particularly in *P. falciparum* infections (WHO, 2016). Several efforts, both at national and international levels, have resulted in reducing malaria incidences. According to WHO (2013); and Cotter, Sturrock, Hsiang, Liu, Philips, Hwang, Gueye, Fullman, Goshing, & Feachem, (2013), prevalence of malaria was reduced by 17% while malaria-specific deaths were reduced by 26% between the years 2000 and 2010. These reductions were made possible by

increased funding, efficient vector control measures, reinforcement of health care systems, better therapeutic approaches and persistent monitoring (WHO, 2013; White, Pukrittayakamee, Hien, Faiz, Makuolu, & Dondorp, 2014;). However, focus on this global agenda has increased towards the control and elimination of this dreaded disease (Feachem, Philips, Target & Snow, 2020).

The failure of existing methods for malaria control has ignited interest in several new approaches which includes the development of genetically modified mosquitoes (GMMs) designed to either reduce population sizes or to replace existing populations with vectors unable to transmit the disease. Most species of mosquitoes do not transmit malaria, and even among species that do, many individuals seem incapable of transmitting the disease, they are refractory. Accordingly, there is reason to hope that the genes that permit malarial infections in mosquitoes can be identified and replaced or altered in terms of their function. In this way, it is hoped that mosquito populations will become refractory to the parasite, eventually leading to malaria transmission being halted. Other methods for generating refractoriness involve using antibodies that kill parasites within the mosquito (Capurro, Coleman , Beerntsen & Myles, 2010) and discovering genes that govern refractoriness in natural populations (Riehle, Markianos, Niare, Xu & Li, 2006). However, driving a refractory construct efficiently through the vector mosquito population such that the population of susceptible mosquitoes will be replaced, still remains problematic (Ribeiro & Kidwell, 2014).

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH), nitric oxide (NO) which results in oxidative stress leading to cellular damage are known as antioxidants. Oxidation is important to many living organisms for production of energy for biological processes. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxi-

dation of cells, leading to cell death and tissue damage. Free radicals are responsible for aging and causing various human diseases such as atherosclerosis, diabetes, cancer, hypertension, Alzheimer's disease, Parkinsonism and cirrhosis (Pal, Ganguly, Tahsin & Acharya, 2010). Antioxidants play an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Sies, 1997). In recent years, natural antioxidants, particularly those present in plants have gained increasing interests among consumers and the scientific community. Reactive oxygen species produce oxidative stress in the cells of human body such that each cell faces about 10,000 oxidative hits per second (Lata & Ahuja, 2013). Frequent intake of fruits and vegetables are associated with a lower risk of age-related disease such as coronary heart diseases and cancer (La Vecchia, Altier, & Tavani, 2011). Recently, there is high demand of natural antioxidants as nutraceuticals and food additives (Kalim, Bhattacharyya, Banerjee & Chattopadhyay 2010). Reative oxygen species when gererated overtakes the antioxidant defense of the cells, the biological systems are attacked by these free radicals and degenerative diseases are developed.

According to Hazra, Biswas & Mandal, (2008) exertion of oxidative stress on human cells by free radicals causes damage of macromolecules such as protein and DNA resulting in pathological processes.

Processes which prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, or prevent mutations are important in the prevention of cancer and other oxidative diseases (Gordon, 1996). Studies have also tested the antioxidant capacity of plants and their constituents *in vivo* (Shruthi, Roshan, Timilsina & Sunita 2013; Alisi, Asiwe, Ene & Alisi, 2018).

Treatment of this parasitic disease still relies on the administration of drugs whose efficacy is constantly frustrated by the emergence of resistant strains of the parasite, coupled with high cost of such orthodox drugs. Consequently, many caregivers, herbal homes and traditional medicine practitioners resort to the use of various medicinal plants in the management of malaria. This practice has become rampant due to the high cost and unavailability of antimalarial drugs, as well as constant emergence of resistant strains of *Plasmodium* species. Herbal or traditional medicines are the first form of treatment given to many patients with suspected symptoms of malaria, especially in developing countries, such as Nigeria, Ghana, Mali and Zambia (Uzochukwu, Ezeoke, Emma-Ukaegbu, Onwujekwe, & Sibeudu, 2010; WHO, 2013).

Medicinal plants have been used in the prevention and cure of malaria in various parts of the world. This is due to the fact that they are inexpensive, readily available, familiar with the peoples' cultural beliefs and practices (WHO, 2008). Prevention, control and elimination measures of malaria are hindered by problems such as economic conditions, poor health care systems, emergence of resistant species of the *Plasmodium* parasite to commercially available antimalarial drugs and environmental factors found in endemic areas (Mills, Lubell, & Hanson, 2018). A combination of these factors has made about 80% or more of the world population, especially people in rural areas, to still depend solely on medicinal plants or traditional medicines around them to meet up with their various health challenges (WHO, 2015). This attitude towards medicinal plants has placed medicinal plants at the center of pharmaceutical research (Kirca & Arsian, 2008). These natural resources also represent the principal sources of natural antioxidants and bioactive agents (Conforti, Sosa, Marrelli, Menichini, Statti, Uzunov & Loggia, 2018).

However, one of the major challenges of using these medicinal plants includes non documentation of these medicinal plants because the knowledge is usually passed on orally from one generation to another. It therefore becomes necessary to correctly identify, classify and scientifically evaluate these natural resources. This might be the only way of creating continued access into the knowledge and techniques of these medicinal plants in order to make meaningful contributions to primary health care.

1.2 Statement of Problem

Malaria remains a killer disease which affects all age groups, particularly children below the age of five years, pregnant women and sickle-cell anaemia patients (Salako, 2014). Despite advances in technology and modern medicine, comprehensive eradication strategies at local and international levels and the huge sums of money voted each year for the prevention and elimination of this disease, it is still prevalent and causes many deaths especially in tropical countries, such as Nigeria. It constitutes a huge burden on Nigeria and other affected nations and retards economic development in these countries. The expenses come in form of costs for prevention, treatment and loss of man-hour or productivity. This economic burden in turn affects the Gross Domestic Product (GDP) of these developing countries due to the high morbidity and mortality rates associated with malaria.

Following the emergence of resistant strains of P*lasmodium falciparum* to artemisinin derivatives (which is the current class of drug used against malaria), there is an urgent need to search for alternative malaria drugs which will be less expensive and readily available.

Although numerous studies have been done on plants with antimalarial properties, very few antimalarial templates emerged from such studies because the bioactive compounds responsible for

the antimalarial activities of such plants were not isolated and characterized. In view of this, the present study was conceived to determine the *in vitro* antioxidant and antimalarial properties of two wild plants; *Chasmanthera dependens* (Hochst) and *Dictyandra arborescence* (Welw.), and ascertain the possibility of using them to mitigate the malaria scourge.

Chasmanthera dependens (Hochst) and *Dictyandra arborescens* (Welw.) are wild plants belonging to the Menispermaceae and Rubiaceae families of flowering plants. Their medicinal uses are scarce and nearly unavailable in literature. However, in some local communities, such as Amuzi in Imo State Nigeria, traditional healers have affirmed that the root and leaf extracts of these plants are used to treat malaria. A scientific study is therefore needed to validate this claim.

1.3 Aim and Objectives of Study

The aim of this research was to study the *in vitro* antioxidant and antimalarial properties of the plants- *Chasmanthera dependens* (Hochst) and *Dictyandra arborescens* (Welw.).

Specific Objectives

(i) To determine the phytochemicals present in the two plants extracts and ascertain their respective roles.

(ii) To determine *in vitro* antioxidant properties of the plants extracts.

(iii) To determine the acute toxicity (LD_{50}) of the crude aqueous and methanolic extracts of the leaves, stems and roots of *Chasmanthera dependens* and *Dictyandra aborescens* plants on male Swiss albino mice**.**

(iv) To determine *in vivo* antimalarial activities of the crude aqueous and methanolic extracts of the leaves, stems and roots of the two plants, using male Swiss albino mice infected with *Plasmodium berghei*.

(v) To isolate the bioactive compound responsible for the antimalarial activity of any extract with the highest antimalarial activity

1.4 Research Hypotheses

The study was guided by the following null hypotheses:

1. The crude aqueous and methanolic extracts of *Chasmanthera dependens* and *Dictyandra arborescens* plants do not have antioxidant properties
2. The crude aqueous and methanolic extracts of *Chasmanthera dependens* and *Dictyandra arborescens* plants do not have antimalarial activities
3. The bioactive compounds present in the plants' extracts are not responsible for their antimalarial and antioxidant activities

1.5 Justification of Study

The outcome of this research work will be of immense benefit in many respects. It is coming at a time of explosion of trado-medicine in Nigeria, as plant–derived drugs are seen marketed in shops, on the streets, offices, radio and television stations, with unsubstantiated claims being made by the producers on the efficacy of these drugs. Worthy of concern is the good number of people who patronize these products without any knowledge of their potency and safety.

Nigeria is blessed with dense vegetation with a wide variety of medicinal plants. These medicinal plants have continued to be used across cultures as sources of valuable medicines for various ailments. They equally play crucial roles in pharmaceutical practices in Nigeria. It is therefore imperative to look closely at these traditional plants to see if using them alternatively with modern medicine can remove the menace caused by malaria.

Since some powerful modern antimalarial drugs owe their origins to plants, e.g quinine from *Cincona officinalis* bark and artemisinin from *Artemisia annua*, active new ingredients could be synthesized from these study plants. Plants contain secondary metabolites which help them to survive adverse environmental conditions, pests and diseases as well as menace from invaders.

The findings of this study will thus, verify the claims by traditional healers that the plants- *Chasmanthera dependens* and *Dictyandra arborescens* actually possess antimalarial agents, perhaps paving way for the isolation of antimalarial compounds from these plants.

1.6 Scope of Study

The scope of this research work included the collection of the leaves, stems and roots of *Chasmanthera dependens* and *Dictyandra aborescens* plants for the determination of phytochemicals, antioxidant activities, acute toxicity, antimalarial potentials, as well as possible isolation of the bioactive compound(s).

CHAPTER II: LITERATURE REVIEW

2.1 Brief History of the Study Plants

2.1.1 *Chasmanthera dependens* (Hochst)

2.1.2 Botanical Classification and Ecology

Kingdom - Plantae

Phyllum - Tracheophyta

Class - Magnoliopsida

Order - Ranunculales

Family - Menispermaceae

Genus - Chasmanthera

Species - *Chasmanthera dependens*

Plate 2.1 Shows the plant-*Chasmanthera dependens*.

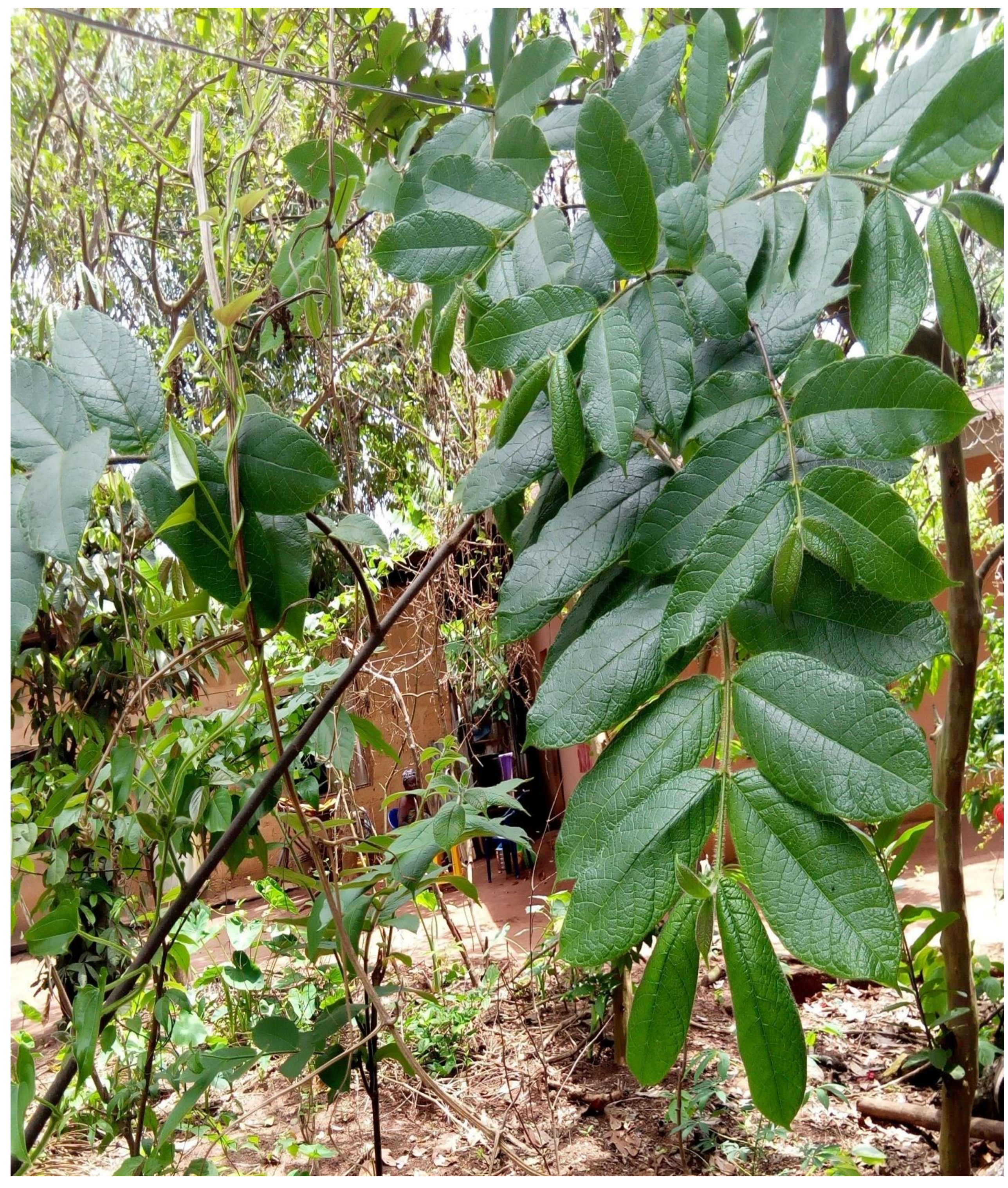

Plate 2.1: *Chasmanthera dependens*

Chasmanthera dependens (Hochst) is a member of the menispermaceae family and is commonly called *Chasmanthera*. It is a woody climber which generally grows around forest margins, savannah or secondary forest. It thrives in moist and dense evergreen forest and grows up to 1500m, preferring well-drained soils. Usually, it is intentionally cultivated in homes or gardens as a medicinal plant. It is called 'aguru' in Igbo (South East Nigeria), and 'atoo' in Yoruba (South West Nigeria).

It is an important medicinal plant used in the treatment of various ailments, such as red-eye infections (Ogunlesi, Okiei, Ademoye, & Ogunlesi, 2008); veneral diseases, convulsions, snakebites, epilepsy, and in management of fractures (Odugbemi, 2008, Ogunlesi *et al*., 2008). It also serves as a general tonic for physical and nervous weakness (Iwu, Duncan, & Okunji 1999). According to Onabanjo, John, Sokale & Samuel (2009) and Morebise, Awe, Makinde, & Olajide (2011), methanolic extract of the dried leaves have anti-inflammatory and analgesic effects on laboratory animals. Similarly, Adekunle & Okoli (2002) reported that the aqueous and ethanolic crude extracts of the leaves have antifungal activity. Githinji, Irungu, Tonia, Rukuga, Mutai, Mathaura, & Wanjoya (2010) reported antimicrobial activities of *C. dependens* stem on fungal yeast, Gram + and Gram – bacteria. *In vitro* antileishmanial and immunomodulatory effects of *C. dependens* stem and leaves against leishmania major promastigotes and infected microphages have been reported by Ogulesi, Okiei, Ademoye & Ogunlesi (2010). Essential oil constituents of the stem have equally been documented (Okiei *et al*., 2009). Bioactive agents isolated from the plant include berberine-type alkaloids, palmitine, colombamine and jateorhizine (Iwu *et al*., 1999). Phytochemical screening of the stem led to the isolation of quaternary alkaloids including pseudocolombamine, magnoflorine and non-phenolic alkaloids, such as tetrahydro palmitine, liriodenine, lysicamine, oxoglaucine, anonaine, nornuciferine, norglaucine and o-

dimethylcorytuberine (Ohiri, Verpoorte, & Baeheim 1982). Iwu *et al*. (1999) also reported that berberine sulphate in the plant inhibits leishmania. A furanoid diterpene, 8-hydroxyl columbine, was also isolated from the bark of *Chasmanthera dependens* as stated by Oguakwu, Galeffic, Nicolletti, Messana & Mariri- Battolo (1986). Local alcohol, mixed with aqueous extract of the leaves, has aphrodisiac effect as (Chukwuma, 2008).

Quadri & Yakubu (2017) studied the fertility activity of aqueous extract of *Chasmanthera dependens* roots in male rats. This study revealed that the aqueous extract of this plant demonstrated pro-spermatogenic, fertility enhancing, and androgenic activities in male rats.

2.1.3 Family: Menispermaceae and their medicinal values

Menispermaceae was coined from two greek words 'mene' (which means moon) and 'sperma' (meaning seed). This is because the seeds of the plants in this family are semi-circular and moon-shaped, and this is a characteristic attribute of members of this family (Jahan, Khatun, Jahan, & Seraj, 2010).

The species in this family are climbing and rarely erect shrubs or small trees. They are dioecious in nature, their 1eaves are usually simple, alternate, often petiolate, rarely trifoliate or palmately lobed. The inflorescence could be cymose, racemose, paniculate, capitulate, and the flowers are small, unisexual and regularly shaped. The fruits are pulpy drupes, while the seeds are often curved and horseshoe-shaped, and may or may not have an endosperm (Jahan *et al*., 2010).

Members of the menispermaceae family are medicinal in nature. As a result, they occupy a central position as sources of medicinal agents. Many of the plants in the menispermaceae family are seen in the tropics and subtropics, although, some genera are found in the warm temperate regions (Trease & Evans, 2002).

Most of the species found in Nigeria are also widespread in other African countries, especially in West African countries where they are seen in evergreen forests. *Chasmanthera dependens,* which is a member of this family, is commonly planted around residential areas and gardens as a medicinal plant.

There are 70 genera in this family and about 420 species. Plants in this family are used in traditional medicine in several countries. Most of them have been reported to contain phytochemicals of therapeutic importance.

Three acetylcholine esterase inhibitors, isolated from a Thai medicinal plant, *Stephania venosa* (used in the treatment of Alzeimer's disease), belong to the menispermaceae family. Stepharanine, cyclanoline and N-methyl stepholidine are the inhibitors and were identified as quaternary protoberberine alkaloids (Ingkaninan, 2006). Alkaloidal extract from the root bark of *Albertisia villosa* contains bisbenzylisoquinoline- cycleanine which is reported to have antibacterial, antifungal, antiplasmodial and cytotoxic activities. This justifies use of this plant in traditional medicine for the treatment of infectious diseases (Lohombo-Ekomba, 2004). The methanolic leaf extract of *Cissampelos mucronata* was reported to demonstrate protective action against indomethacin-induced ulcer in rats (Nwafor & Akah, 2003). Antinociceptive and anti-arthritic activity has equally been reported for *Cissampelos pareira* roots (Amresh, 2007). Antihelminthic activity of aporphine alkaloids from *Cissampelos capensis* against *Haemonchus contortus* has been reported (Ayers, 2007). According to Su (2004), the ethyl acetate extract of stem of *Macrococculus pomiferus* inhibited cyclooxygenase-2. Shirwaiker, (2005), also reported that the alcoholic stem extract of *Coscinium fenestratum* possessed anti-diabetic activity. This conclusion was made when the effect of the extract was studied in streptotozin-nicotinamide induced type 2 diabetic rats.

2.1.4 Dictyandra arborescens (Welw.)

2.1.5 Botanical classification and Ecology

Kingdom – Plantae

Phyllum – Tracheophyta

Class – Magnoliopsida

Order – Gentianales

Family – Rubiaceae

Genus – Dictyandra

Species – *Dictyandra arborescens*

Plate 2.2 shows the plant-*Dictyandra arborescens*

Plate 2.2: ***Dictyandra arborescens***

Rubiaceae is a family of flowering plants called the madder or coffe family. The family took its name from the madder genus: *Rubia*. Other plants, such as gardenia, cinchona, gambier, ixora, nauclaceae, and theligonaceae, have also been included in the family. Rubiaceae has about 630 genera and more than 13000 species in the family. This makes the rubiaceae one of the six largest angiosperm families; inclusive are asteraceae, orchidacaeae, fabaceae, poacaeae and euphorbiaceae in terms of number of genera and species. Rubiaceae species are usually found in warm and tropical climates (Dalziel, 1997). Rubiaceae has a wide variety of growth forms. Shrubs are the most common, but members of the family can also be trees, or herbs. Species are mainly woody; but less than 20% of the genera are herbaceous. A large number of them grow in Sub-saharan Africa. The most represented include anthospermeae, morindeae, spermacoceae, cinchoneae, naucleae, coffeae, gardineae, and pavatteae (Karou, Melanbega, Chebara, Moardo, & Trease, 2007).

2.1.6 Ethnomedicinal uses of plants in the rubiaceae family

Rubiaceae species have an extensive patronage in traditional medicine. They are continuously screened for their pharmacological properties. Due to their wide distribution, plants in this family are used globally as ornamentals, foods and medicines. The most popular members of the family are the two species of shrub, *Coffea canphora* (also called *Coffea robusta*) and *Coffea arabica*, which are used in the production of coffee. Numerous other species from the genus are also seen in horticulture. The genus: *Ixora* is made up of plants that are cultivated in warm climate gardens.

Plants of the genus: *Cinchona* have attracted great attention in medicine due to their contained alkaloids; the most recognized one is quinine, the first efficacious therapeutic agent in treating malaria.

Many plants in the rubiaceae family have been reported to possess antihypertensive, antimalarial, antimicrobial, antidiabetic, antioxidant and anti-inflammatory activities. Bioactive compounds found in plants of this family include indole alkaloids, terpenoids and anthraquinones. Presence of these alkaloids in some rubiaceae family members has been documented. Phytochemical analysis of *Canthium horridum* was reported by Yang, Zuo, Han, Wang and Bian (2012). Extracts of *Canthium multiflorum* revealed presence of these chemical compounds, alkaloids, terpenes, and tannins as reported by Akomo, Zongo, Karou, Obume, Savadogo, Ateke & Traore, (2009). Chemical consituents of the stems of *Canthium simile* were studied by Wang, Chen, Yin, Han, & Zhang, (2007) and their study revealed the presence of saponins, terpenoids and flavonoids. Wabo & Mpoame (2011) reported nematicidal activity of extracts of the bark of *Canthium mannii* plant. They contain valuable secondary metabolites such alkaloids and saponnins. Ajayi, Kadiri, Egbedi, & Oyeyemi, (2011) reported presence of alkaloids, tannins, saponnins, reducing compounds and flavonoids in *Retigynia umbellulata*.

Borreria and Spermacoce have alkaloids, iridoids, flavonoids and terpenoids (Conserva & Ferreira, 2012). Phytochemical screening of *Canthium multiflorum* leaves equally recorded presence of phytochemicals, such as saponnins, tannins, flavonois, alkaloids, proanthocyanidines, anthracenosides, coumarins, terpenoids, sterols and carotenoids (Akomo *et al*., 2009). Antimicrobial activities of plants in this family may be due to the wide range of compounds they synthesize. Akomo *et al*. (2009) reported potent antimicrobial activity of *Canthium multiflorum* against *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, *Proteus misrabilis*, *Staphylococcus aureus*, *Staphylococcus camorum*, *Shigella dysentrica* and *Salmonella enterica*.

Asase, Kokubun, Grayer, Kite, Simmonds, Yeboah, and Odamtten, (2008) reported inhibition of growth both of gram positive and gram negative bacteria by the acetone extract of *Mitragyna*

inermis. Adomi (2008) reported high zone of inhibition using aqueous extract of *Morinda lucida*. The ethanolic extract of the wild plant *Canthium coromandelicum* showed a broad spectrum of antimicrobial activity against *Salmonella typhi* and antifungal activity against *Candida albicans* (Sathish, Shanmugam, Palvannan, & Kumar, 2018).

Borreria spermacoce species of rubiaceae, as well as their isolated compounds, exhibit biological activities which include anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer and hepatoprotective activities. Alkaloids and iridoids are the major active principles reported (Conserva & Ferreira, 2012). Members of the Rubiaceae family represent valuable source of new secondary metabolites for therapeutic purposes. Crude extracts of these plants were reported to have antibacterial activity. Some plants in this family have also been reported to have anticancer and hepatoprotective activities (Simplice, 2011).

2.1.7 Antiplasmodial effects of some plants from the rubiaceae family

Antimalarial activity is the biological property that has received great attention by scientists who are interested in rubiaceae plants. Extracts of these plants were tested on parasite cultures *in vitro* to determine their viability on the main malarial parasite- *Plasmodium falciparum*. Parasites are usually isolated from untreated malaria patients or obtained as reference chloroquine sensitive *P. falciparum.*

Many rubiaceae plants are used in traditional medicine as antimalarial agents. This is because the main antimalarial drug, quinine is obtained from the rubiaceae family. Researchers are therefore optimistic that similar compounds with comparable properties may occur in other members of the family.

Crude extracts of many rubiaceae plants have been tested with success on *P. falciparum*. Benoit-Vical, Valentino, Cournac, Pelimier, & Bantile, (2018) reported IC_{50} of 0.6 µg Ml^{-1} using aqueous extract of *Nauclea latifolia* on the Columbian multidrug resistant *Plasmodium falciparum* (CQRPf FcBI). However, the ethanol extract of the same plant gave IC_{50} of 8.9 µg ml^{-1} on the same Columbian CQRPf strain as reported by Zirihi, Mambu, Guede-Guina, Bodo, & Grellier, (2005).

2.2 Epidemiology of the Disease

Incidence of malaria originally was thought to be more prevalent in the rural areas where environmental conditions favour parasite transmission (McMicheal, Haines, Sloof & Kovats, 2006). However, there is considerable risk of infection in urban areas. This is due to increased number of slums, resulting in increased malaria transmission in urban areas of developing countries, especially Nigeria. Swamps, gutters, thick vegetation in urban areas, farming in residential areas contribute significantly to the breeding of mosquitoes. This is made possible by practices, such as irrigation, ponds for fish farming, as well as storage water tanks for livestock. These provide excellent breeding grounds for mosquitoes. A combination of these factors could put farmers at high risk of malaria, a disease that has serious adverse consequences on agricultural productivity. Greenwood, Fidock, Kyle, Kappe, Alonso, Collins, & Duffy, (2008) stated that the disease majorly affects poor people in tropical and subtropical areas, where climatic conditions are appropriate for development of vectors and parasites. Majority of cases of malaria are reported in Africa, especially Nigeria (WHO, 2008 and 2015).

Those at high risk of malarial infection are children under five years of age and pregnant women (WHO, 2016). Approximately 40% of the world population, particularly those living in poor countries, is at risk of malaria. This category of people is mostly found in the tropical and

subtropical regions of the world. At least 300 million acute cases of malaria occur globally each year resulting in more than 1 million deaths. Approximately 90% of these deaths occur in Sub-Saharan Africa (SSA) (American Association for the Advancement of Science (AAAS, 1991; WHO, 2011).

Malaria is also responsible for 40% of public health expenditure, 30-50% of in-patients admission and up to 50% of outpatient visits in all malaria-endemic areas (WHO, 1998; WHO, 2011).
An estimated 655,000 people died globally in 2010 as a result of malaria, 86% comprising of children under the age of five (WHO, 2011).

Out of the 627,000 deaths recorded in Africa in 2012 as a result of malaria, 482,000 were children under the age of five (WHO, 2013; WHO, 2014). In 2013, malaria killed 437,000 children under the age of five in sub-Saharan Africa (WHO, 2014). However, according to World Health Organization (2014), death rates due to malaria reduced by 54% in African region between the years 2000 and 2013. The incidence further reduced to about 34% in the African Sub region (WHO, 2016). Unfortunately, malaria cases increased globally from 217 million in 2016 to 219 million in 2017 resulting to about 53% malaria deaths in 7 African countries, and only Nigeria accounted for 19% of the deaths (WHO, 2016). Despite the substantial efforts to control and eliminate malaria, disease incidence and consequent deaths still continue, serving as a serious reminder that war against this dreaded disease is yet to be defeated. In Nigeria, malaria is still a major public health challenge, where it causes more deaths than in any other country of the world (WHO, 2015). According to WHO (2015) estimate, approximately 100 million cases of malaria exist in Nigeria, with over 300,000 deaths per year. This figure compares with 215,000 deaths which occur in Nigeria as a result of HIV/AIDS (WHO, 2011). Death toll as a result of mosquito

bites therefore remains one of the greatest tragedies of the 21st century. Reasons for the persistence of malaria in the African continent, especially in Nigeria, may not be far-fetched as most of the infections are caused by *P. falciparum* which is the most harmful of the four human malaria parasites (AAAS, 1991, WHO, 1998). The malarial parasite vector (female *Anopheles* mosquito) is widely spread in the African continent and very difficult to control (WHO, 2012, World Health Report, 2012). The areas of malaria transmission globally are shown in Fig. 2.1.

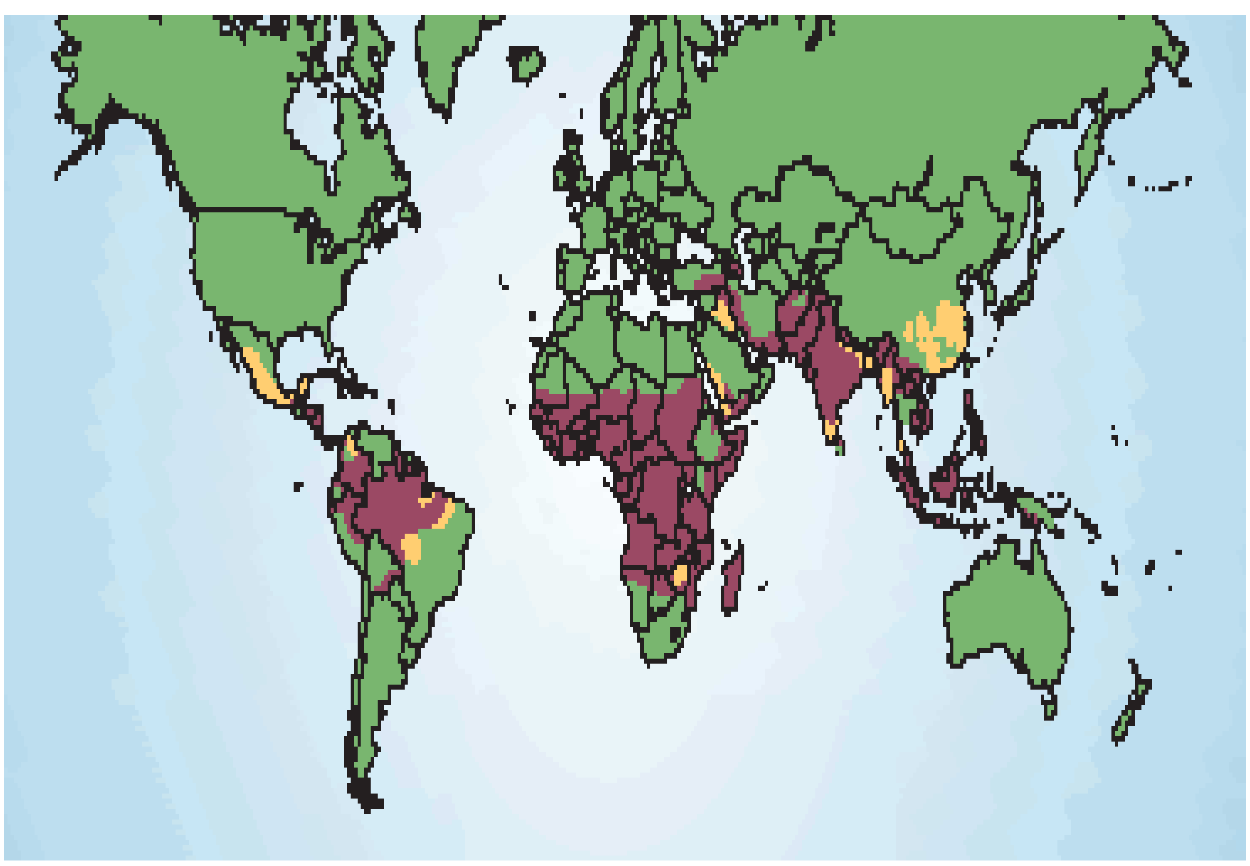

Figure 2.1: World map showing areas of malaria transmission

Source: (Toure & Oduola, 2004)

- Areas where malaria transmission occur
- Areas with limited risk
- No malaria

2.3 Life Cycle of Malaria Parasite

Malaria parasite enters the human host when an infected female *Anopheles* mosquito feeds on human blood. Inside the human host, the parasite undergoes a sequence of changes to complete its complex life-cycle.

The life cycle of the parasite is quite complex and may differ according to the plasmodia species involved. This distinctive life-cycle has been adapted to man over time. In general, the *Plasmodium* parasite has three reproductive phases thus: the exoerythrocytic or pre-erythrocytic phase which takes place in the liver; this is followed by the erythrocytic phase which takes place in the red blood cells or erythrocytes; finally the sexual phase which takes place in the stomach of the mosquito (Fig.2.2) (Ekpenyong & Eyo, 2006).

Inside the female mosquito, sexual reproduction occurs and is known as sporogony. This then produces sporozoites which are injected from the salivary gland of an infected mosquito and from there into the blood stream of man during feeding of the mosquito. Thirthy (30) minutes after bite, the sporozoites leave the blood stream and enter the parenchyma cells of the liver (hepatocytes). Here they reproduce asexually via a process referred to as exoerythrocytic schizogony. The nucleus of the parasite undergoes repeated division which gives rise to thousands of uninucleated mezoroites (Afolabi, 2001). Between 6 and 16 days of infection, the nucleus of the liver is displaced, the liver cells containing the schizonts rupture and the merozoites enter the circulation. If the infection is due to *P. falciparim* or *P. malariae*, the tissue schizonts rupture almost at the same time and all the merozoites leave the liver. On the contrary, *P. vivax* and *P.ovale* have two types of exoerythrocytic types. There is a primary type that develops and ruptures within 6-9 days; while in the other secondary type, the merozoites may remain dormant in the liver for weeks,

months or even years before development begins. This therefore results in a relapse of erythrocytic infection. Unlike other *Plasmodium* species, gametocyte appearance is delayed in *P. falciparum,* making acute recurrence common in clinically cured individuals (Abdul –Gani & Beira, 2014).

The exoerythrocytic phase usually kicks off when an infected female *Anopheles* mosquito feeds on human blood and transfers the parasite.

During the erythrocytic phase, merozoites released from the tissue schizonts enter the blood stream to attack the red blood cells or erythrocytes. This occurs when the liver cells burst, and merozoites then enter the blood stream where they attack the red blood cells. Other pigments and waste products are liberated, together with the merozoites. These are responsible for the feverish condition associated with the parasite attack. The merozoites of the different *Plasmodium* species show preference for erythrocytes at specific ages (Ekpenyong & Eyo, 2006). While the merozoites of *P. vivax* attack young erythrocytes, those of *P.malariae* attack the adult ones; whereas those of *P. falciparium* randomly attack any available red blood cell. This explains why *P. falciparum* is responsible for most malaria cases. About 3-15 days after the manifestation of symptoms of malaria, some merozoites differentiate into sexual forms, i.e female macro gametocytes and male micro gametocytes.

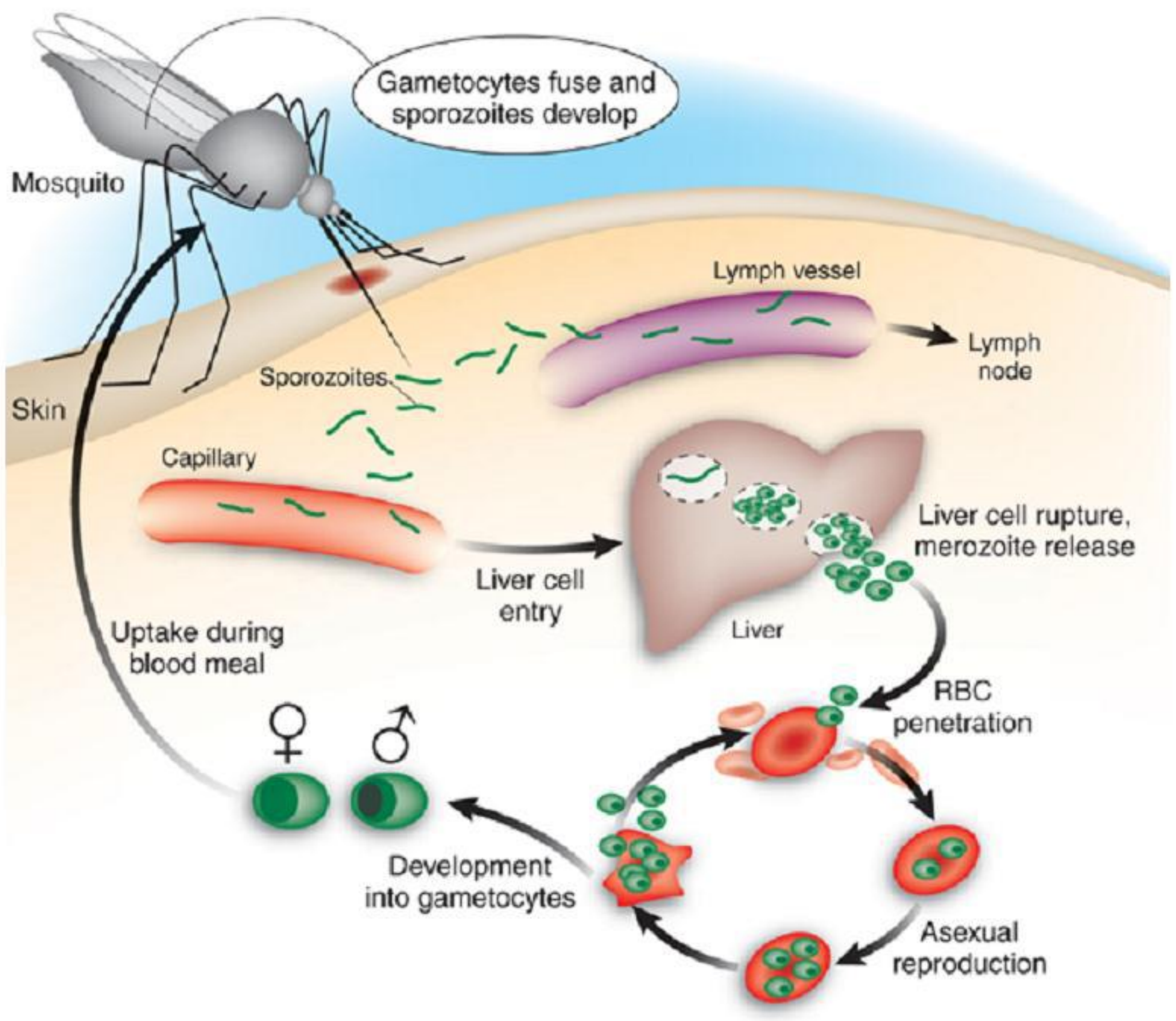

Figure 2:2: Life cycle of the Malaria Parasite.

Source: (Batista, De-Jesu Silva Junior, & De Oliveira, 2009).

The sexual phase of the life cycle of *Plasmodium* starts when the mosquito ingests the gametocytes with a blood meal from an infected person. This then undergoes additional development in the abdomen of the mosquito. The microgamete moves actively forward through small projection that forms on the female parasite. Fertilization occurs and the zygote elongates to become motile ookinetes (fertilized eggs). These ookinetes force their way between the epithelial cells of the outer

surface of the mosquito stomach to form oocyst. According to Philips (2001) and Heelan & Ingersoll, 2002), the oocyst increases gradually in size as the nucleus divides continuously giving rise to sporozoites. When the mature sporocyst ruptures, the sporozoites are freed into the body cavity where they find their way to the salivary gland. As the mosquito feeds on the blood of a new host (man), the sporozoites are injected into the blood thereby initiating a new schizogonic cycle as reported by Ekpenyong & Eyo (2006).

At different stages of development, the exterior proteins and metabolic pathways of the parasites are modified; these enable these parasites to escape clearance. According to Lymbery & Thompson (2012), this equally makes it difficult for effective drugs and vaccines to be developed.

2.4 Prevalence, Transmission Indices and Pathology of Malaria

According to WHO (2015), incidence of malaria in a particular area is determined by the population of mosquitoes, conducive environment that supports the breeding of mosquitoes, number of people that have gametocytes within their red blood cells, efficacy of anti-malarial measures as well as use of suppressive drugs. Other factors, such as parasite species and strain, economic situations, human population, climatic conditions etc, can also affect prevalence of malaria in a given geographical area. These factors are equally responsible for the level of malaria endemicity and immunity development. Those at high risk of malaria infection include people who are exposed to the parasite, either as visitors to or those living in malaria endemic areas. Munguti (1998) equally stated that it is mostly the under privileged in these areas that are more at risk.

A visit to the paediatrics or out-patient wards of many hospitals in tropical Africa reveals that malaria is still the main cause of morbidity and mortality, especially in children (Greenwood,

1999). This disease has been established as a major killer in Sub-Saharan Africa. It is more prevalent in the rainy seasons (Imboua-Bogui & Diawara, 2006). According to the duo, 45% of children in the rain forest zones suffer from malaria more during the rainy season compared to 12% reported in dry season.

Afolabi, Salako, & Mafe, (1997) and FMoH (2010) reported that malaria is holoendemic in all parts of Nigeria, accounting for about 30% of child mortality. This can be explained from the point of view of microclimate, topography, population densities, cultural practices and general ways of life. Intensity of transmission and management of the disease is largely affected by these parameters. According to Federal Ministry of Health (FMoH, 2008 & 2014), their target was to reduce malaria related deaths to as much as 50% by the year 2013. This aim is yet to be achieved as the disease still remains prevalent. Owing to this, studies on prevalence of malaria and other studies based on malaria surveys are imperative for accessing the efficacy and impact of malaria control measures and programmes at all levels (FMoH, 2015). Similarly, a total of 6,132 children, aged between 6 months and 12 years presented with pyrexia illness, diagnosed clinically as malaria, were screened for parasitaemia (Salako, Ajayi, Sounwi, & Walker, 2000). This study revealed that, 2,930 (47.8%) of the children tested positive to the asexual forms of the malarial parasite. Ninety-seven percent of the cases was *P. falciparum,* while 3% was for mixed infection of *P. falciparum* and *P. ovale*, then less than 1% was *P. malaria*e alone.

WHO (2015) also reported that percentage of new cases of malaria has declined by 25% globally, and mortality rate fell by 42%. Several measures adopted to combat this menace are not enough to accomplish total eradication, and the price paid for maintaining these interventions now run into several billion dollars annually. To worsen the situation, the malaria parasite continues to develop

resistance to available drugs, thus these resistant strains spread (Olaseinde, Ojurongbe, Akinjogunla, Egwari, & Adeyeba, 2015).

In Nigeria, malaria transmission occurs throughout the year, in both urban and rural areas (Anumudu, Okafor, & Afolabi 2006; FMoH, 2014). This problem is aggravated by rural-urban migration, persistent poverty, environmental degradation, lack of access to decent housing, as well as portable water and sanitation problems. These factors encourage risks of malaria infection and parasite resistance through inconsistent malaria treatment options.

Transmission of malaria in Nigeria varies according to climatic, geographic and socio-economic conditions. This is due to the fact that Nigeria is a large and diverse country. According to Awolola, Okwa, Hunt, Ogunrinade, & Coetzee, (2002) and Awolola *et al*. (2003), many regions of the country do not have adequate or consistent data on vector control, and there is equally very little information on sporozoite rates in southern Nigeria. Most information on malaria transmission in Nigeria comes from irregular studies carried out in the northern parts of the country (Wagbatsoma & Ogbeide, 1995). In southern Nigeria, a study on mosquito populations was carried out by Onyabe & Conn (2001). They reported distribution of two major malarial parasites, *Anopheles gambiae* and *Anopheles arabienisis*, in southwestern Nigeria. Wagbatsoma & Ogbeide (1995) and Awolola *et al*., (2002) studied population dynamics and biting behavior of anophelines in this area. Awolola *et al*., (2002) focused their study on *A. funestus* group. According to Okwa, Akinmolayan, Carter, & Hurd, (2016), mainly *Anopheles* species are considered to be involved in transmission in Nigeria. Victims of malarial infection, who show no symptoms remain an ongoing source of transmission. In their study, Griffin, Hollingsworth, Okell,

Churcher, & White, (2010) stated that in spite of increasing efforts to control the disease, the burden of malaria still remains high in Nigeria as a result of constant high level of transmission.

When malarial parasites find themselves in the blood stream, they contaminate and destroy red blood cells (erythrocytes). Strickland (2008) reported that damage to these vital cells results in fever and flu-like symptoms, such as chills, headache, muscle aches, tiredness, nausea, vomiting as well as diarrhoea. In cases of uncomplicated malaria, the symptoms are non-specific and may include fever. Malaria is not complicated when no clinical or laboratory signs appear to indicate disease intensity or vital organ failure, although symptoms may be present.

P. falciparum is responsible for severe malaria which may result in severe breathing difficulties, low blood sugar, anaemia or coma, if the disease is not given immediate attention. Children are mostly at risk because of their low level of immunity to the dreaded parasite (WHO, 2000).

Patients may show symptoms of infection, such as drowsiness, irritability, lack of appetite and inability to sleep. Chills may follow after these symptoms, leading to development of fever characterized by very fast breathing. According to Davis (2011), the temperature of the body returns quickly to normal and the patient experiences sweating as the fever subsides. Appearance of symptoms may vary depending on the previous immune status and age of the individual (Jayant & Mani, 2010).

Symptoms of malaria can re-emerge after varying symptom-free periods. Reappearance can be classified as 'recrudescence or relapse' depending on the cause (White *et al*., 2014). In recrudescence reappearance, the symptoms come back after a symptom-free period. This is usually caused by parasites persisting in the blood as a result of inadequate or ineffective treatment. On the other hand, symptoms of relapse re-occur after the parasites must have been eliminated from the

blood, but persist as dormant hyponozoites in the liver cells. Relapse is usually common in *P.vixax* and *P.ovale* attacks (Nwaozor, 2016).

Symptoms of malaria may appear 9-14 days after the bite of an infectious mosquito according tothe American Association for the Advancement of Science (AAAS, 1991). However, symptoms may appear much later in individuals who had taken anti-malarial drugs earlier. Early signs of the disease, such as septicemia, gastroenteritis and viral diseases, which are common to all malaria species, are similar to flu-like symptoms and may be like other conditions. Symptoms include headache, fever, shivering, pains at the joints, vomiting, haemolytic anaemia, jaundice, haemoglobin in the urine, retinal damage as well as convulsions. A severe symptom of malaria is ‘paroxysm’ which refers to a cyclical occurrence of sudden coldness, followed by shivering, fever and sweating. This occurs every two days in *P. vivax* and *P.malariae* attacks. In the case of *P. falciparum*, which is accompanied by severe symptoms and deaths, symptoms may appear 9-30 days after an infection. In cases of cerebral malaria, the victim may suffer neurological symptoms, such as abnormal posturing, nystagmus, conjugate gaze palsy (failure of the eyes to turn together in the same direction), seizure or even coma. Malaria also has other deadly consequences which may include respiratory distress that occurs in up to 25% of adults and 40% of children with severe *P.falciparum* malaria. These are possibly caused by respiratory compensation of metabolic acidosis, non-cardiogenic pulmonary oedema, concomitant pneumonia and severe anaemia (Nwaozor, 2016).

Periodic paroxysms, which occur in malaria, are directly associated with the events in the blood stream. Initially, there is chill, which persists for about 15 minutes to 1 hour. This begins when uniformly dividing generations of the parasites rupture their hosts’ red blood cells and break away

into the blood. During this period, symptoms, such as nausea, vomiting, headache and shivering may be experienced. Subsequent hot session, which lasts for a number of hours, is characterized by serious fever. Throughout this period, the *Plasmodium* parasites most likely attack new red blood cells. Sweating stage concludes the series of events. The fever slows down and the patient sleeps and feels fairly well when he wakes up. Early periods of infection normally marks asynchronous cycles during which the fever is uneven, and paroxysms may occur irregularly at 48-72 hours intervals, however, *P. falciparum* pyrexia may persist for 8 hours or more and may go beyond 41ºC. Progressively, splenomegaly and hepatomegaly will appear (Peters & Pasvol, 2002). Every organ is prone to malarial attack, thus symptoms also vary accordingly (Jayant & Mani, 2010).

2.5 The Role of Climate in the Spread of Malaria

The life cycle of malaria parasites can be influenced by climatic conditions. Climatic conditions thus, play a major role in the geographic distribution as well as transmission of malaria. Intense rainfall favours breeding of mosquitoes; it generates breeding sites which enhance development of the parasite. This developmental process takes place only in damp areas which is made possible by availability of rainfall. However, in the absence of rainfall, these breeding sites are destroyed; they can also be disrupted or shattered by extreme rainfall (Mouchet, 1998).

As the mosquito reaches adult stage, it is favoured by suitable temperature and humidity; steady rainfall determines their rate of survival. Successful transmission of malaria parasite depends on the ability of the female *Anopheles* to live long enough when they must have been infected through blood meal on an infected host. This period allows the parasite they ingested to complete their growth. This cycle lasts for between 9 and 12 days. Warm ambient temperature reduces period of the extrinsic cycle /transmission. On the other hand, below an optimum temperature, the extrinsic

cycle may not be completed (Mouchet, 1998). Transmission of malarial parasite is higher in warmer regions of the world (tropical and semi-tropical areas, and low altitudes particularly for *P. falciparum*). Wilson (1991) reported that present trends of global warming could add to the geographic range of malaria and may become responsible for malaria epidemics.

Climatic factors, such as temperature and rainfall, also influence human behaviors which expose them to mosquitoes. Increase in temperature in hot seasons supports habit of sleeping in the open. It also prevents individuals from using insecticide treated nets, where they are available. According to Mouchet (1998), in hot season, particularly during the harvest season, farmers are forced to sleep in open fields, without any form of protection against mosquito bites.

2.6 Orthodox Medicines Used in the Treatment of Malaria and their Mode of Action

Treatment of this parasitic disease still relies on the administration of drugs whose efficacy is constantly frustrated by the emergence of resistant strains of the parasite.Drugs are used for malaria prevention, in the form of chemoprophylaxis; these act to suppress the blood stage of malarial infection. In the treatment of malaria, the aim is usually to stop acute infection in the blood, to take care of clinical symptoms, to get rid of hypnozoites in the liver, to avert potential relapses as well as prevent multiple infections (Schlitzer, 2000 and Rudrapall, 2011). Treatment comprises of radical and curative processes. Pharmacological drugs used are numerous and they include: Chloroquine, Mefloquine, Quinine (from *Cinchona* bark) and Primaquine. Others include Pyremethamine, Artemisinin derivatives (artesunate, artemether, arteether) and amino alcohols, such as Lumefantrine and Halofantrine, Sulfadoxime, and Doxycyclines (Singh, 2011). Jayant & Mani (2010) opined that appearance of drug resistant strains of the parasites, which often results in treatment failure in many cases, poses a major challenge associated with malaria treatment.

Antimalarial drugs are used for presumptive or prophylactic treatment, and prophylactic treatment implies that the drug is taken as a preventive measure to fend off the disease. For instance, since the groups at high risk are pregnant women and children below the age of 5 years, regular prophylaxis in women and children will reduce mortality rate. The aim of the therapy includes: to wipe out hepatic schizonts before they release merozoite cells which will attack red blood cells, destroying the erythrocytes blood schizont before the merozoites are released, as well as destroying the gametocytes thereby preventing them from being taken up by mosquitoes during a blood meal. On the other hand, presumptive treatment is that given to patients who are suspected to have malaria already. Treatment is based on signs observed by the physician and symptoms shown by such patients. It is not based on symptoms observed through diagnosis by blood film. The drug used ought to be curative with a single dose, without any side effect and suitable for such individual.

Treatment of malaria had involved the following drugs: Chloroquine, quinine, mefloquine and halofantrine, antifolates, atavaquone and artemisinin and its derivatives.

2.6.1 Chloroquine

Chloroquine ($C_{18}H_{26}CIN_3$) has a molecular weight of 320 and was synthesized in 1934 as Resochin (chloroquine) and Sontochins (3-methyl- chloroquine) by German Scientists who were trying to discover a substitute for quinine (Akman, Cerman, Yemice & Kazokolu, 2011). It is an amphilic weak base with two fused aromatic rings that have conjugated bonds. It crosses the cell membrane with relative ease (Ferrari & Cutler, 1991; Sundelin & Terman, 2001). It binds to nucleic acids by electrostatic, hydrogen and Van der Waal's forces (Parker & Irvin, 2002).

The compounds synthesized (Resochin and Sontochins) were members of a new class of anti malarial, the four-amino quinolines. After the 2nd World War, Chloroquine and Dichlorodiphenyltricholoroethane (DDT) became the two major tools in WHO's universal eradication of malaria programme. Unfortunately, chloroquine- resistant *P. falciparum* emerged in four different locations. According to Medicine for Malaria Venture (2014), it started with Thai-Cambodian border to Venezuela and some parts of Colombia around 1960; in mid 1970s, it spread to Papua New Guinea, and down to Africa in 1978 (Kenya and Tanzania). By 1983, it had spread to Sudan, Uganda, Zambia and Malawi.

Chloroquine was not originally known as an effective antimalarial drug till the 1940s for the duration of the World War II. By 1946, it was discovered to be of better-quality than other existing synthetic antimalarials hence it started being used as a specific antimalarial therapy (Klinger, Morad, Westal, Laskin, Spitzer, Koren & Buncic 2001). Chloroquine thus became a major breakthrough antimalarial chemotherapy for the next 40 years. It speedily became the drug of choice internationally to tackle uncomplicated *P. falciparum* infections. It was later adopted to be part of the Global Malaria Eradication programme launched by the WHO in 1955. Chloroquine can either be taken as a prophylactic or for presumptive treatment.

Mechanism of action of Chloroquine

Several research works have been carried out on the mechanism of action of chloroquine (Foote & Cowman, 1994; Foley & Tilly, 1997; Peters, 1997; Klinger *et al*., 2001; Akman *et al*., 2011). Chloroquine, as an antimalarial drug, inhibits biosynthesis of DNA and RNA thus inducing rapid degradation of ribosomes. Wallace, Gudsoorkar, Weisman, & Venuturupalli, (2012) proposed that the inhibition of DNA replication is the common mechanism of action of chloroquine. This drug

builds up in very high concentrations in the food vacuole of the parasite. Inside the food vacuole, the drug probably inhibits the detoxification of heme. Chloroquine equally gets protonated ($CQ2^{+}$) because of the acidic nature of the digestive vacuole (pH 4.7) and afterwards it finds it difficult to leave the vacuole through diffusion. Chloroquine caps hemozoin molecules and stops further biocrystallization of heme. This leads to heme buildup. Chloroquine binds to heme (or FP) to form FP-chloroquine complex. This complex is highly toxic to the parasite cell because it disrupts membrane function. Actions of the toxic FP-chloroquine complex and FP result in cell death. This consequently leads to the auto-digestion of the parasite cell. In essence, the parasite cell drowns in its own metabolic products.

2.6.2 Quinine

Quinine is obtained from the bark of a South African tree. It was believed that it first came to Europe through a Countess who was treated with it in Peru in the 1600s (Medicine for Malaria Venture, 2014). In 1742, the bark of the plant from where quinine was obtained was named *Cinchona* by Linnaeus. Then in 1820, quinine was isolated from the *Cinchona* bark by two French Chemists. It was the active ingredient of the bark, isolated and purified. This drug became a global reference drug for the cure of severe and uncomplicated fever in many therapeutic regimens. Till today, quinine is used in treating malaria, notwithstanding intermittent observations of quinine resistance (Medicine for Malaria Venture, 2014).

Another derivative of *Cinchona* bark, quinimax (a combination of quinine, quinidine, and cinchonine), is also used (Rynes, 2007). According to Bjorkman, Willcox, Marbiah, & Payne, (1991); and Krishna & White, (1996), quinine is usually administered at least 7 days to non-immune patients but it is more effective in immune patients if it is given for 3 to 5 days. This is

because its effectiveness depends on the potential immune system of the host (Miller, Greenberg, & Campbell, 2009). In the United States where quinine is not commercially accessible, its D-isomer, quinidine is used. Quinine is also used together with antibiotics, such as tetracycline or doxycycline.

Mode of action of quinine

Quinine and chloroquine act in a similar manner but with some variations. While chloroquine acts by clumping the malaria pigment, quinine does not act this way (Wallace, Gudsoorkar, Weisman, & Venuturupalli, 2012). Additionally, quinine, being a weaker base than chloroquine, has less affinity for heme. This means that mechanisms apart from ion transport and heme-drug interactions are necessary for the action of these drugs (Foley & Tilly, 1998). According to Chou, Chevli, & Fitch, (2008), quinine equally interacts rather weakly with heme but is recorded to inhibit polymerization of heme (Slater 1992; Chou & Fitch, 1993), and heme catalase activity (Ribeiro, Miller, & Paganga, 1997). Without a specific transporter, quinine is likely to build up less efficiently in the food vacuole than chloroquine. More research work is still needed to determine the closeness the mode of action of quinine to that of chloroquine.

2.6.3 Mefloquine and Halofantrine

The United States Army resorted to mefloquine in 1970s as an answer to the progressive poor treatment rates of chloroquine (Medicine for Malaria Venture, 2014). Its development was a collaborative effort of the US Army medical research and development team, WHO/TDR and Hoffman-la Roche, Inc. The clinical trials began in 1972 (Kaufman & Krise, 2007). Mefloquine is noted for its long half-life (10.3 to 20.5 days) in malaria patients, and in healthy persons (13.8 to 27.5 days) (Karbwang & White, 1990; Na-Bangchang, Molunto, Banmairuroi, Thanavibul, &

Karbwang, 1995). Mefloquine is recommended as a prophylactic and therapeutic remedy in chloroquine-resistant areas. There have not been potential neuropsychiatric side-effects of mefloquine (Choo, 1996). Parasite resistance to mefloquine started appearing in Asia in 1985, at a time the drug was already accessible to people (Medicine for Malaria Venture, 2014).

Halofantrine, a tricyclic compound, was developed almost at the same time with mefloquine. It was mostly utilized as a second-line agent. The reason may be due to its cardiotoxic side-effects and unpredictable pharmacokinetics.

Mode of action

Wallace *et al*., (2012) reported a relatively weak interaction between mefloquine and free heme, with dissociation constant (Kd) values ranging from 3.3×10^{-7} to 1.63×10^{-5} M. Mefloquine inhibits heme polymerization *in vitro* (Slater & Cerami, 1992; Slater, 1993) with a comparable or lesser effectiveness than chloroquine (Chou & Fitch, 1993; Raynes, Foley, Tilley, Deady, 1996). Due to the lower basicity of mefloquine, it is less likely that it would attain the intravacuolar concentration needed to inhibit heme polymerization. Moreover, when chloroquine was used in treating *P. berghei* infected mice, there was a decrease in hemozoin production, whereas mefloquine and quinine had no such effect (Chou & Fitch, 1993). Sugioka & Suzuki (2001) opined that mefloquine was also a much less potent enhancer of the peroxidase activity of heme than chloroquine. It is reported that it obstructs capability of chloroquine to enhance heme-induced cell lysis (Dutta & Fitch, 2003). Available data implies therefore that, mefloquine does not obstruct same step in the parasite-feeding process like chloroquine does (Arden & Kolb, 2006).

2.6.4 Antifolates

Many extensively used antimalarial drugs are grouped together under the Folate class; their function in malaria control is hindered by quick appearance of parasite resistance (Plowe *et al.*, 2008). When the enzymes of the folate pathway are inhibited, there is a consequent decrease in pyrimidine synthesis. Thus, there is reduction in DNA, serine, and methionine formation. Activity of these antimalarials is exhibited at all growing stages of the asexual erythrocytic cycle and on immature gametocytes.

Mode of action

Generally, antifolate drugs are inhibitory to either of the enzymes dihydrofolate reductase (DHFR) (pyrimethamine, cycloguanil) or dihydropteroate synthase (DHPS) (sulfadoxine). These enzymes play key roles in the biosynthesis of folate. According to Saifi, Beg, Harrath, Altayalan & Al-Quraishy (2012), when this metabolic pathway is inhibited, there is inhibition in the biosynthesis of pyrimidines, purines, and other amino acids. Antifolate antimalarial drugs are known to obstruct folate metabolism, an essential pathway for malaria parasite survival.

2.6.5 Atovaquone

The second world War marked discovery of the antimalarial activities of hydroxynaphthoquinones. Atovaquone is the initial active compound in this class of antimalarials. Presently, it is sold as Malarone, a combination of fixed amounts of atovaquone plus proguanil.

Mode of action

With a fixed combination of proguanil, atovaquone {2-[trans-4-(40-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone}, hydroxynaphthoquinone is used in the treatment, as well as prevention of malaria. Although it is known to be principally active on mitochondrial, its mode of

action, and cooperation with proguanil, are yet to be fully understood. This case is made worse by the varied functions of mitochondria in different organisms, and difficulties encountered during experiments. According to Fry & Pudney (1992), it is usually believed that atovaquone works on the mitochondrial electron transport chain; however, currently, its action and cooperation with proguanil is attributed to its obstruction of mitochondrial membrane potential. Atovaquone interferes with the activity of cytochrome-C reductase in *P. falciparum*. Atovaquone is an analogue of ubiquinone which binds to the cytochrome *bc1* complex of the parasite. In the opinion of Plowe *et al*., (2008), the parasite mitochondria electron transport chain gets rid of electrons produced by dihydroororate dehydrogenase in the synthesis of pyrimidines. Interference of this process by atovaquone probably kills the parasite (Ruiz-Irastoza, Egurbide, Pijoan, Garmendia, Villar, Martinez-Beriotxoa, Erdozain & Aguirre, 2006). In recent times, it has been demonstrated in *P. yoelli* that atovaquone also disperses the potential of mitochondrial membrane of the parasite and this might kill the parasite by introducing a process comparable with apoptosis.

2.6.6 Artemisinin and its derivatives

A major challenge facing elimination of malaria is the development of resistance to classical quinoline antimalarial compounds, such as chloroquine and antifolates (Woodrow & Haynes, 2005). Extracts from a wormwood, *Artemisia annua,* have been used for several years in China to cure fevers. Some Chinese scientists isolated the bioactive ingredients in this plant in 1970 (Saifi *et al*., 2012). Artemisinin and its derivatives, such as artesunate, artemether, and arteether, are widely used in China and Southeast Asia where resistance to quinoline - containing drugs and to antifolate drugs are very high (Meshnick, Taylor, & Kamchonwongpaisan, 2006). The currently used artemisinin-type compounds are either the natural extract artemisinin or its semi-synthetic derivatives (dihydroartemisinin, artesunate, artemether). Till date, there is a higher reduction rate

of parasites per cycle when arteminisin is used than any other drugs (White, 2007). Artemisinins are not only effective against multi-resistant strains of *P. falciparum*, they also have wide range specificity against the plasmodium life cycle. This includes activity throughout the asexual blood stages and the sexual gametocyte stages. These reduce spread of the disease in areas with low transmission (Terkuile, White, Holloway, Pasvol & Krishna, 2013).

Artemisinin Combination Therapies (ACTs) are currently the most effective cure for malaria. A more current and recent drug is Coartem, which was developed in 1994, and combines artemisinin with lumefantirine (Medicine for Malaria Venture, 2014). Coartem, which was designed by Chinese scientists, not only kills the parasite, it also stays long in the blood to help prevent any resistance by the malaria parasite (Oyeniyi, 2009)

Mode of action

Although the exact mode of action of artemisinin is not known, it is believed that the endoperoxide bridge, pharmacophore, facilitates development of various classes of totally synthetic endoperoxides (Vennerstrom, Arbe-Barnes, Brun, Charman, Chiu, Chollet, Dong, Dorn & Charman, 2004). When taken, the artemisinin derivatives are quickly hydrolyzed to the biologically active metabolite, dihydroartemisinin. Present information on mode of action of artemisinin is recorded by Meshnick, Arbe-Barnes, Brun, Charman, Chiu & Chollet, (2006) and Cumming *et al*. (2007). Artemisinin has an unusual structure. Its activity depends on the occurrence of endoperoxide bond. Foote & Cowman (2014) reported that molecules without this bond do not have antimalarial activity. On interaction with iron or heme, the endoperoxide bonds probably decompose into free radicals (Meshnick *et al*., 2006; Paitayatat, Tarnchompoo, Thebtaranonth, & Yuthavong, 2007). In contrast to other redox reactions, this process cannot be reversed; therefore a single drug molecule produces a single free radical. However, effect of these

free radicals on the malaria parasite is yet to be fully elucidated. Since the amount of free radicals is not adequate to cause general membrane damage, Meshnick (2003) opined that a 'specific free radical target' exists. According to Zhang & Gerhard, (2008); Creek, Ryan, Charman, Chiu, Prankered, Vennerstrom, & Charmans, (2009) artemisinin free radicals can form covalent bonds with either heme or other parasite proteins; and an earlier assumption was that a heme-artemisinin compound may inhibit production of hemozoin. Although no evidence of decreased quantities of hemozoin in cultures of artemisinin-treated *P. falciparum* has been recorded, artemisinin binds to 6 specific *P.falciparum* proteins; one of these proteins belongs to the family of translationally monitored tumor protein but the exact consequence of this protein alkylation on the malaria parasite is still to be ascertained (Asawamahasakda, Ittarat, Chang, McElroy, & Meshnick, 2004; Ramacher, Umansky & Efferth, 2009).

2.7 Medicinal Plants and Control of Malaria in Nigeria

Since the history of man, medicinal plants have been found very useful across all cultures as important sources of medicine (Hoareau & Dasilva, 1999). Various medicinal plants play crucial roles in health care delivery system in Nigeria. Different plant parts continue to be essential sources of therapeutic agents to fight severe health issues globally. Pharmacognostic examinations continue to be carried out on plants to discover new drugs or templates for the production of new therapeutics. According to Philipson (1991), studies on the chemical compounds contained in medicinal plants are imperative. This is because most drugs, such as quinine and artemisinin used in the treatment of malaria, were obtained from plants. Due to the emergence of resistant strains of many disease pathogens, for instance malaria parasites, towards well known or recognized drugs, researchers seek for alternative drugs from plants. It is a general belief that if herbs or plants used by our ancestors so many years ago did not prove efficacious, diseases, such as malaria, would

have ravaged Africa. Emergence of resistant parasites to antimalarial drugs, particularly in third-world countries that already have poor economic indices, necessitated discovery of novel drugs or drug combinations for prevention and cure of malaria. These new drugs must have novel modes of action, and must probably be chemically different from already existing ones (Philipson & Wright, 1991). Other factors that have contributed to the increasing interest in medicinal plants include: high prices of orthodox drugs and low therapeutic index of synthethic compounds (Seed, 2000).

Almost 80 % of the world population believe in the efficacy of herbs and medicinal plants for their health care needs (Satish & Ranjana, 2013). In Nigeria, use of different medicinal plants for treatment of different ailments has been in practice for a long time. Across cultures, a variety of medicinal plants are used. This practice is traditionally handed down to generations all over the globe. Trust in medicinal plants is mainly because they are safe, effective, affordable, readily available and culturally preferred. Activities of these medicinal plants are either identified by instinct/insight, prior knowledge or even trial and error. Globally, traditional medicine practitioners use different medicinal plants for treatment of malaria; however, this practice is yet to be fully documented. Knowledge of traditional medicine practice and medicinal plants is presently passed on from one generation to another through oral information. Nigeria is blessed with rich flora diversity and has a large number of plant species which have been acknowledged to have antimalarial activities.

The first known antimalaria in history was extracted from the bark of *Cinchona*. *Cinchona* is a member of the Rubiaceae family; one of the alkaloids, quinine, is still widely used. Infusions from the plant bark were the main treatment for human malaria since 1632 (Baird, Caneta-Miguel, Masba, Bustos, Abrenica, Layawen & Wignall, 1996). After some years, quinine was isolated and

characterized (Saxena, Pant, Jain, & Bhakuni, 2003). Consequently, it became the most ancient and central antimalarial drug used for malaria treatment. *Artemisia annua* is another old medicinal plant of great importance. It was rediscovered in China in the 1970s as a significant source of the antimalarial drug, Artemisinin (Bruce-Chwatt, 1982; Klayman, 1985). Artemisinin-combination therapies (ACTs) have been officially accepted as first-line treatment of uncomplicated malaria in Nigeria since 2005 (Mokuolu, Okoro, Ayetoro, & Adewara, 2007). Use of ACTs is however limited, owing to its high cost, inadequate adherence to Good Manufacturing Practices (GMP) in the production of artemisinin derivatives and toxicity (Afonso, Hunt, Cheesman, Alves, Cunha, Rosario & Cravo 2006; Boareto, Muller, Bufalo, Botelho, De- Araujo, Foglio & Dalsenter, 2008).

Both *Cinchona* plant and *Artemisia annua*, from where most effective drugs (quinine and artemisinin) were isolated, are not indigenous to sub-Saharan Africa where malaria is endemic. Plants in the tropical rainforest are known to have more concentrations of natural chemical defences and a greater diversity compared to plants from other biomes; they therefore represent possible sources of new medicines (Balick, Elizabetsky & Laird, 2016). Research on plants from this region is therefore encouraged particularly as malaria accounts for most deaths in this region.

Nigeria is richly blessed with rich flora diversity and most of these plant species are extensively used by indigenes within a locality for medicinal purposes. A lot of medicinal plants are used for treatment of malaria in the Southern part of Nigeria. This is due to the presence of thick rain forests and humid tropical climate, which favour persistent malaria transmission throughout the year. Some of these medicinal plants are used by all ethnic and cultural groups in the country for the treatment of malaria; examples include *Alstonia boonei* and *Azadirachta indica* (neem plant).

Alstonia boonei is a high tree of about 33 m; has a straight and fluted stem, but no buttress roots. Species of this plant are highly valued, particularly where affordable antimalarial drugs are not effective, due to drug-resistant malaria parasites. The stem, bark or leaves of this plant are administered as decoction or 'teas'. It is often used as an ingredient in malaria 'steam therapy'. In recent times, tablets have been formulated from the stem bark extract and made accessible as an antimalarial drug (Majekodunmi, Adegoke & Odeku, 2008).

Members of Meliaceae family are also generally used for treatment of malaria in Nigeria. These include: *Azadirachta indica*, *Khaya senegalensis* and *Khaya grandifoliola*. *Azadirachta indica* (neem plant) is also used in other African countries as a remedy against fever and/or malaria. The tree is an evergreen tree which grows up to 25 m in height; it is widely used in traditional medicine practice in different forms, such as aqueous decoction of the leaves, stem bark and roots (Obih and Makinde, 1995). According to Isah, Ibrahim & Iwalewa (2003), the efficacy of this plant in malaria control in Nigeria led to efforts to manufacture tablet suspensions of the bark and leaves. This exhibited high prophylactic, moderate suppressive and a very minimal curative schizonticidal effect in mouse model of malaria. *Khaya senegalensis* and *Khaya grandifoliola* are often planted along the roads for purposes of providing shade. Decoctions from their stem barks are widely used as antimalarial medication but they appear to have adverse effects (Adebayo, Yakubu, Egwim, Owoyele & Enaibe, 2013; Bumah, Essien, Agbedahunsi & Ekah, 2015).

Morinda lucida, another member of the Rubiaceae family, is also extensively used for treatment of malaria in Nigeria. It is a tree of about 9–18 m, and bears a dense crown of slender crooked branches (Avwioro, Aloamaka, Ojianya, Oduola & Ekpo, 2005). The aerial parts, stem bark or root bark, of *Morinda lucida* are generally used in West Africa for treatment of malaria and other

tropical diseases. Makinde, Awe, & Salako (2004) reported a seasonal variation in its antimalarial activity. Another plant in this family (Rubiaceae), *Nauclea latifolia,* is a small spreading tree whose aqueous decoction of the root bark proved effective against malaria.

Numerous other plants are used for treatment of malaria in Nigeria. For instance *Quassia amara* and *Quassia undulata* (Simaroubaceae), are widely used in southwest Nigeria (Phillipson & Wright, 1991). *Quassia amara,* often called bitterwood tree, is 2–6 m in height, and has a high antimalarial activity. It is used both for curative and preventive purposes. *Enantia chlorantha* (Annonaceae), an ornamental tree, of about 30 m in height, with dense foliage and spreading crown, is another plant that is used against malaria. The stem bark is usually used against fever/malaria by traditional medicine practitioners in the rain forest regions. *Carica papaya* (Caricaceae), commonly called pawpaw, is widely known and planted in the tropics for its edible fruit. The leaves are also used as weak decoction against malaria. *Fagara zanthoxyloides* (Rutaceae) is another medicinal plant whose root is widely used as chewing stick in Nigeria and West Africa at large (Odebiyi & Sofowora, 2009). Aqueous extract of the root of this plant is used for malaria treatment by the local people. *Spathodea campanulata* (Bignoniaceae), generally known as 'African tulip tree', is a tropical tree of Africa, although it has now adapted to other tropical regions around the world, due to its ornamental value. It is used as a remedy against malaria in southwestern Nigeria by drinking the decoction of its stem bark.

Majority of these antimalarial plants are used singly (monotherapy); only a few plants are taken as combinations. An example of this combination therapy is the multi-herbal extract called ‘Agbo-Iba’ by the Yorubas. It comprises the leaves of *Cajanus cajan* (pigeon pea), *Euphorbia lateriflora*, *Cymbopogon giganteus, Cassa alata, Nauclea latifolia Mangifera indica* leaf and bark, and *Uvaria*

chamae bark (Nwabuisi, 2002). Another example is multi-herbal combination made from a mixture of the leaves of *Carica papaya*, *Cymbopogon citratus*, *Anacardium occidentale* and *Azadirachta indica* and used in 'steam therapy', where the patients are covered with a thick blanket for them to inhale the vapour from the boiling pot. Investigation of these medicinal plants as an important aspect of research may provide solution to malaria chemotherapy whose major challenge is to discover safe and effective drugs whose potency will not be compromised by *plasmodial* resistance (Watsierah & Ouma, 2014; White, Pukrittayakamee, Hien, Faiz, Makuolu & Dondorp, 2014). Table 2.1 shows medicinal plants used in South East Nigeria against malaria while Table 2.2 shows combination of medicinal plants used in same area against malaria.

Table 2.1: Medicinal plants used against malaria in South East Nigeria

Plant name(family)	Common/Local name	Parts Used	Method of preparation
Nauclea latifolia Rubiaceae	African peach, Mburumuilu	Stem bark and Roots	a. Boil roots in water for 2 hours. b. Soak overnight in water or alcohol, take the filtrate half glass cup, three times a day.
Azadirachta indica Meliaceae	Neem tree, Dogonyaro	Stem bark and Leaves	Boil leaves in water, allow to cool, then filter, take half glass cup, one in the morning and in the night.
Cymbopogon citratus Poaceae	Lemon grass, Achara ehi	Leaves	Boil dried grasses for 30 minutes. Allow to cool and sieve Take full glass cup, one in the morning and in the Night
Morinda lucida Benth Rubiaceae	Brimstone tree, Ezeogu	Leaves, Stem Bark	a. Boil leaves and stem bark for 10 minutes, sieve and take half glass cup, twice daily. b. Soak stem bark in ethanol for 24 hours, sieve and take half glass cup, once daily.
Sida acuta Burn. F Malvaceae	Broom weed, Udo	Leaves, Tender stems	Boil leaves and stem bark for 30 minutes, sieve and take half glass cup, twice daily
Ocimum gratissimum. L Lamiaceae	Scent leaf, Nchuanwu	Leaves	Wash and squeeze two hand-ful of fresh or dried leaves with water not more 2 big drinking cups. Take the juice half cup, three times day. Discard the remaining
Alstonia boonei De Wild Apocynaceae	Stool wood, Egbu	Stem bark, Root and Leaves	a. Boil all plant parts in water for 30 mins and allow to stand overnight. Then take half glass cup twice a day. b. Soak roots and stem bark in alcohol over night and strain. Then take half glass cup twice a day.

Carica papaya L. Caricaceae	Paw-paw	Seed, leaves	a. Boil fresh or dried matured leaves with ground seeds. Sieve and take one glass cup, twice daily for 3 days. b. Boil the leaves and inhale the steam under a covered blanket. c. Boil the leaves for few minutes and bath the warm water
Mangifera indica Anacardiaceae	Mango, Mangoro	leaves	Boil leaves in water, allow to cool, then filter and take half glass cup, one in the morning and at night.
Vernonia amygdalina Del. Asteraceae	Bitter leaf, Olugbu	leaves	Wash and squeeze leaf in a basin of water for 5 minutes. Discard water, repeat process, filter and drink half cup, twice daily.
Psidium guajava L Myrtaceae	Guava, gova	leaves	Boil leaves in water, allow to cool, then filter and take half glass cup, one in the morning and at night.

Source: Ogbehi & Ebong, 2015

Table 2.2: Combination of medicinal plants used against malaria in South East Nigeria

Botanical Names of the plants in the Concoction	Family Names	Local Names	Plant parts used
a.*Anthocleista nobilis* b. *Nauclea latifolia* c. *Napoleona imperialis*	a. Loganiaceae b. Rubiaceae c. Lecythidaceae	a. Okpokoro b. Mburumuilu c. Nnekeloche	a.Root b. Root c. Stem bark, Root
a.*Acalypha wilkesiana* Muell Arg b. *Carica papaya* L. c. *Enantia chlorantha* Oliv	a. Euphorbiaceae b. Caricaceae c. Annonaceae	 b. Poo-poo c. Dokita Igbo	a. Leaves b. Leaves, seeds c. Stem bark
a. *Nauclea latifolia* b. *Alstonia boonei* De Wild *c. Picralima nitida* Stapf	a. Rubiaceae b. Apocynaceae c. Apocynaceae	a. Mburumuilu b. Egbu c. Osi-igwe	a.Root b. Root c. Seeds, fruit rind
a. *Anacardium occidentale* L. b. *Carica papaya* L. c. *Cymbopogon citratus* *d. Azadirachta indica*	a. Anacardiaceae b. Caricaceae c. Poaceae d. *Meliaceae*	a. Cashuu b. Poo-poo c. Achara-ehi d. Dogonyaro	a. Stem bark, Leaves b. Leaves c. Leaves d. Leaves
a. *Solanum nigrum* L b. *Morinda lucida* *c. Khaya grandifoliolia*	a. Solanaceae b. Rubiaceae c. Meliaceae	a. Anara ugumakpe b. Ezeogwu c. Oganwo	a. Leaves, whole fruits b. Leaves, stem bark c. Stem Bark Leaves, whole fruits
a. *Strophantus hispidus* b. *Acioa barteria* c. *Magnifera indica* *d. Carica papaya*	Osisi kaguru *b.* Ogbodo *c.* Mangoro *d.* Paw paw		a. Roots b. Roots c. Leaves d. Leaves

Source: Ogbehi & Ebong, 2015.

2.8 Factors that Support Use of Medicinal Plants in the Treatment of Malaria

Although there have been many innovations in orthodox drugs, medicinal plants or herbal therapies are widely patronized for treatment of malaria all over the world, particularly in Nigeria. Reasons for this patronage include the following:

2.8.1 Resistance of malarial parasites to antimalarial drugs

According to WHO (2015), antimalarial drug resistance is defined as the ability of a strain of parasite to survive and reproduce regardless of the administration and absorption of drugs given in doses equal to or higher than those usually recommended within the tolerance of the subject. Resistance to antimalarial drugs is now one of the major challenges facing eradication of malaria today as opined by Olaseinde, Ajayi, Taiwo, Adekeye & Adeyeba, (2015). Resistance of *P. falciparum* to ACTs in Cambodia poses a risk to the world's malaria control and eradication efforts (Ariey, Witkowski, Amaratunga, Beghain, & Langlois, 2014). The trend continues as ACT resistant parasites spread from Western Cambodia to greater Mekong sub-region and to Africa, as it happened formerly with other antimalarials, such as chloroquine and sulphadoxine/ pyrimethamine. This emerging trend is particularly worrisome. Some possible factors are responsible for increase in the spread of drug resistance; they include economics, human behaviours, pharmacokinetics, as well as the biology of vectors and parasites. Antimalarial drug resistance has been reported in all classes of anti-malarial drugs, even artemisinin derivatives. This poses a major threat to the control of malaria. Resistance has been documented for *P. falciparum, P. malariae* and *P. vivax.* There is considerable variation in the geographical distribution and rates of spread of malaria parasites.

Chloroquine was the first antimalarial drug but is no longer recommended for treatment of malaria due to incidence of resistance reported in 1959 (Talisuna, Langi, Bakyaita, Egwang, Mutabingwa, Watkins & D'Alessandro, 2002). Resistance to chloroquine prepared the stage for the discovery of new antimalarial drugs. Antifolate combination, sulfonamide/pyrimethamine, became the next antimalarials to be used. When resistance was discovered in this class of antimalarials, quinine and its derivatives, such as mefloquine, amodiaquine, as well as related lumefantrine, halofantrine were immediately adopted. Resistance to antimalarials started in Asia and spread to South America, to South Asia. It is now rapidly spreading in the Tropics (Wernsdorfer & Walther, 2003).

Responding to the antimalarial drug resistance problem, WHO came up with a fresh treatment combination therapy policy for *P. falciparum* malaria in all the countries that experienced resistance to monotherapeutic drugs, such as chloroquine, sulfadoxine/pyrimethamine, amodiaquine etc. This involves use of artemisinin and its derivatives (Artemisinin-based Combination Therapy (ACT). ACTs are presently the most effective drug against multi-resistant malaria parasites. ACT has, no doubt, played a major role in reducing the number of deaths due to malaria for over one decade (WHO, 2014). However, according to Rogers, Sem & Tero, (2009), Phyo, Nkhoma, Stepniewska, Ashley & Nair, (2012); Hien, Thuy-Nhien, Phu, Boni & Thanh, (2012); Kyaw, Nyunt, Chit, Aye & Aye, (2013); Ariey, Witkowski, Amaratunga, Beghain & Langlois, (2014), *P. falciparum* resistance against ACT has recently been documented in Thialand-Cambodian border in Asia.

When there is severe malaria, intravenous or intramuscular route is employed for antimalarial drug administration, such drug as quinine or ACT, which can be given in combination with some antibiotics such as clindamycin or doxycycline, tetracycline (WHO, 2010).

Treatment failure is related to antimalarial drug resistance. Inability to clear malaria parasites or clear clinical symptoms, following drug administration, could be as a result of non- potency and not drug resistance because not all cases of treatment failure is as a result of drug resistance. Factors that contribute to treatment failure include: incorrect dosage, non-adherence to the duration of dosage, poor quality of drug, drug interaction, as well as improper diagnosis (WHO, 2010). Each of these factors plays a role in malaria treatment failures in Nigeria and there is need for them to be studied in an effort to eliminate the disease.

2.8.2 Availability and accessibility of anti-malarial drugs in health facilities

Many rural areas have no access to good health care facilities. Most times, these health care centers which are poorly equipped, do not have good roads to access them. Even when there are access roads, the required drugs are hardly available or are inadequate for treatment of malaria (Carrington, 2001). Usually, Public Primary Health centers, especially in rural areas in Nigeria, lack essential facilities. These include antimalaria drugs which most times are either not available, out-of-stock, or close to expiry dates. This problem is aided by improper funding of malaria control programmes, incompetent health personnel, inadequate storage facilities as well as embezzlement of funds meant for acquiring essential anti-malaria drugs. Sometimes when these drugs are available, they are still inaccessible to the poor who are mostly in need of them. This may be due to high cost of the effective drugs, such as ACTs, consultation fees, protocols in getting doctors attention, few health workers to serve the needs of the large population. Other reasons include industrial actions which most times result in worker strikes and the habit of absenteeism on the part of some public primary health care workers. To worsen the situation, most of these health care facilities are not very near to the people, who do not have any means of transportation. These protocols do not come into play in traditional medicine practice. Medicinal

plants or herbs used are accessible and readily available to patients at all times. Kadiri, Adekunle & Ayodele, (2010) stated that in rural areas, where accessing orthodox medical care is hard to get, information on herbal medicine is normally shared. Medicinal plants are gathered from nearby bushes as soon as they are needed. Herbalists are consulted for more serious ailments. These herbalists collect plants which are used for curing known ailments, prepare and administer them and charge a fee which is usually lower than what is charged in hospitals or health centers. People in urban areas equally go to herbal homes or patronize vendors of herbal drugs (Kadiri, 2010). These drug vendors hawk and dispense drugs which have already been prepared from plants to treat different ailments.

2.8.3 Cost of Orthodox Antimalarial Drugs

Aluko (2005) stated that as the economy continues to worsen, many people, especially low income earners and rural dweller, are forced to patronize traditional medicine practitioners and herbalists because they represent a cheaper and accessible means of taking care of their health. Artemisinin Combination Therapies (ACTs), which are the most effective anti-malaria drugs, cannot easily be afforded by many Nigerians, especially the rural dwellers. This is due to their high cost and absence of Government intervention to subsidize them.

With this high cost, many Nigerians are left with no other option than patronizing herbal homes. They, therefore, resort to cheaper herbal remedies whose costs are usually less than those of orthodox drugs, and are obtained locally and naturally within the environment.

2.8.4 Cultural Beliefs

Culture influences the understanding of health issues and illness behavior. This influence on the belief system on conception of illness and disease affects the therapeutic choice and preference to

health facilities (Adepoju, 2005). Some people from particular religious affiliation seek competent health care providers, other religious groups do not believe so. Traditional health care system is largely affected by cultural beliefs as stated by Adepoju (2005). This is so because some steps involved in the procurement of medicines have connection with supernatural beings, especially spiritual practices, such as prayers, sacrifices, charms and incarnations. To this people, disease means anything that affects the body both physically and spiritually.

According to Adepoju (2005), most of the steps involved in the preparation and administration of some traditional medicines are spiritually inclined, and medicines are believed to derive their power from physical and spiritual entities. African medicine is regarded as being spiritual in form and relies mainly on the belief systems of the practitioners. For instance, in African ethnomedicine, the natural and spiritual causes of diseases are acknowledged.

Incantations are equally used to treat ailments and ward off evil. This may be done with or without applying charms and other medicinal preparations. These beliefs are basically sacred and strictly adhered to by a particular cultural group. This runs through the traditional health care system providing a very strong backup for the use of medicinal plants (Adepoju, 2005).

Jegede (2008) stated that patronage of modern health care facilities is better understood within the cultural context of the people. Some still believe that most diseases are better treated with local herbs and plants because modern medicine may not be able to take care of any disease caused by evil spirits. They believe that hospital can do little or nothing about such diseases. This belief system controls the health care seeking behaviour of the people and consequently affects their modern health care patronage.

2.9 Phytochemical and Antioxidant Properties of Medicinal Plants

Phytochemicals refer to non-nutrient plant chemical compounds or bioactive components. It was coined from the greek word "*phyto*" meaning 'plant'. They are also known as phytoconstituents and are accountable for protecting plants against microbial infections and infestations by pests (Doughari, Human, Bennade & Ndakidemi, 2009). According to Doughari & Obidah (2008) and Doughari *et al*. (2009), phytochemicals have been isolated and characterized from fruits, such as grapes and apples, vegetables such as broccoli and onion, spices, such as turmeric, beverages, such as green tea and red wine, etc.

Plants have tremendous potentials to synthesize aromatic substances. Most of these aromatic substances are phenols or their oxygenated-substituted derivatives. Research has shown that these substances consist mainly of secondary metabolites (Doughari *et al*., 2009).

Plant secondary metabolites, most times, help the plant to fight microorganisms; in other words, they serve as defense mechanisms against microbial or insect attacks etc. Terpenoids, for instance are responsible for odours in plants; quinines and tannins are responsible for plant pigmentation, while others are responsible for plant flavor. There is no doubt therefore that valuable medicinal phytochemicals can be derived from these plant secondary metabolites.

Phytochemicals may act by inhibiting microorganisms, inhibition of metabolic processes or modulation of gene expression and signal transduction pathways (Surh, 2003). Phytochemicals are either chemotherapeutic or chemo preventive agents. Chemoprevention refers to using agents to inhibit, reverse, or retard pathogenesis. Extracts from plants and essential oils have demonstrated various modes of action against pathogensis. These include interfering with the phospholipid bilayer of the cell membrane; consequently permeability increases resulting in loss of cellular

constituents, destruction of enzymes that play a role in the production of cellular energy and synthesis of structural components, as well as destroying or inactivating genetic materials. Broadly, mode of action of phytochemicals is taken to be destruction of cytoplasmic membrane, coagulation of cell contents among other actions (Kotzekidou, Giannakidis & Boulamatsis, 2008). The secondary metabolites of plants are as listed below:

2.9.1 Alkaloids

Alkaloids are the largest group of secondary chemical constituents of plants. They are largely made up of ammonia compounds containing nitrogen bases; they are synthesized from amino acid building blocks and have a variety of radicals substituting one or more of the hydrogen atoms in the peptide ring, most having oxygen. Alkaloids have basic properties and are mainly alkaline in reaction, turning red litmus paper blue. In reality, basicity of an alkaloid is contributed by one or more nitrogen atoms that are present in an alkaloid, typically as 1°, 2° or 3° amines. Degree of basicity differs significantly. According to Sarker & Nahar (2007), these variations depend on the structure of the molecule, as well as presence and location of the functional groups. On reacting with acids, they form crystalline salts without producing water (Firn, 2010). Many alkaloids exist in solid forms, such as atropine; some exist as liquids containing carbon, hydrogen, and nitrogen. A good number of alkaloids are highly soluble in alcohol but are moderately soluble in water. Their salts are usually soluble. Alkaloids generate strongly bitter solutions with a solvent. These nitrogenous compounds play crucial roles in defending plants against pests and pathogens. They are also extensively exploited as pharmaceutical agents, stimulants, narcotics, and poisons due to their effective biological activities. Naturally, alkaloids exist abundantly in seeds and roots of plants, and sometimes as a mixture with vegetable acids. Pharmacological applications of alkaloids include as anesthetics and as CNS stimulants (Madziga, Sanni & Sandabe, 2010). About 12,000

alkaloids exist in about 20% species of plants but only a few have been explored for pharmaceutical purposes. Names of alkaloids end with the suffix *–ine* and plant-derived alkaloids clinically used include morphine and codeine (analgesics), tubocurarine (muscle relaxant), sanguinafine and berberine (antibiotics) and vinblastine (anticancer agent). Others include antiarrythmic ajmaline, the pupil dilator atropine, and the sedative scopolamine. Other important plant alkaloids include the addictive stimulants caffeine, nicotine, codeine, atropine, morphine, ergotamine, cocaine, nicotine and ephedrine.

2.9.2 Glycosides

Generally, glycosides are said to be the condensation products of sugars (including polysaccharides) with many different varieties of organic hydroxy (occasionally thiol) compounds (monohydrate in character), such that the hemiacetal entity of the carbohydrate must basically take part in the condensation (Firn, 2010). Physically, glycosides are colorless, crystalline carbon, hydrogen and oxygen-containing (although others contain nitrogen and sulfur) water-soluble phytoconstituents, found in the cell sap. According to Kar (2007) & Firn (2010), glycosides contain carbohydrate (glucose) and non-carbohydrate components (aglycone or genin). Alcohol, glycerol or phenol symbolize aglycones. Glycosides are easily hydrolyzed into their various components with mineral acids and ferments.

Classification of glycosides is based on the type of sugar component, chemical nature of aglycone as well as therapeutic action. Older minor names of glycosides generally have the suffix ‘in’. These names basically include source of the glycoside. Table 2.3 shows examples of glycosides and their sources.

Table 2.3: Examples of glycosides and their sources

Glycoside	Source
Strophanthidin	Strophanthus
Digitoxin	Digitalis
Barbaloin	Aloes
Salicin	Salix
Cantharidin	Cantharides
Prunasin	Prunus

Source: Firn (2010)

Systematic names are however coined by substituting the 'ose' suffix of the parent sugar with "oside".

This class of drugs are normally taken to improve appetite and help digestion. Glycosides are usually found in plants of the Genitiaceae family. They contain entirely bitter compounds that are not chemically related but have the general characteristic of strongly bitter taste. These bitter components act on gustatory nerves, resulting in increased flow of saliva and gastric juices. Lactone group are part of the bitter principles; these may be diterpene lactones (e.g. *andrographolide*) or triterpenoids (e.g. *amarogentin*). A number of these bitter principles either serve as astringents owing to the presence of tannic acid, as antiprotozoan, or serve to reduce thyroxine and metabolism. Examples of glycosides include cardiac glycosides (which act on the heart), anthracene glycosides (purgative, treatment of skin diseases), chalcone glycoside (anticancer), amarogentin, gentiopicrin, andrographolide, ailanthone and polygalin. Sarker & Nahar (2007) opined that plant extracts contain cyanogenic glycosides used as flavouring agents in many pharmaceutical companies. Cancer has been treated with the glycoside, amygdalin. It also serves as a cough suppressant in many preparations.

Ingesting excess cyanogenic glycosides is very dangerous. Sarker & Nahar (2007) reported that foods containing cyanogenic glycosides cause poisoning (severe gastric irritations and damage). Test for O-glycosides involves boiling the plant samples with hydrochloric acid (HCl) or water (H_2O) in order to hydrolyse the anthraquinone glycosides to individual aglycones, adding an aqueous base, such as NaOH or NH_4OH solution. Plant samples containing C-glycosides, are hydrolysed using $FeCl_3$/HCl, together with an aqueous base, such as NaOH or NH_4OH solution. If both solutions result in a pink or violet colour in the base layer after addition of the aqueous base, it becomes an indication of presence of glycosides in such plant sample. Kar (2007) reported use of glycosides as antioxidants or free radical scavengers.

2.9.3 Flavonoids

This group of secondary metabolites form an important group of polyphenols extensively distributed among the plant flora.

Their structure is made up of more than one benzene ring (a range of C_{15} aromatic compounds). These compounds are obtained from the parent compounds known as flavans. More than 4,000 flavonoids exist. Many of them are pigments in higher plants. Examples of flavoids include quercetin, and kaempferol which are present in nearly 70% of plants. Others are flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones and cathechin and leucoanthocyanidins. These compounds are synthesized by plants and are usually rapidly secreted in response to microbial infection. They have also been reported to be efficacious antimicrobial agents.

2.9.4 Phenolics

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical substances that occur widely as natural colour pigments responsible for colour of fruits in plants.

They are mainly synthesized from phenylalanine through action of Phenylalanine Ammonia Lyase (PAL). They play crucial role in plants, due to their numerous activities. Defending plants against pathogens or predators seem to be their most important function. Consequently, they are used against human pathogenic infections (Puupponen- Pimiä, Nohynek, Ammann, Oaksman-Caldentey & Buchert, 2018). Phenolics are classified into (i) phenolic acids (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolies.

Caffeic acid is the most universal of phenolic compounds in plants. Another one is chlorogenic acid which is responsible for allergic dermatitis among humans (Kar, 2007). Most natural antioxidants are phenolic compounds; they are used as nutraceuticals, and are abundant in apples, green-tea, and red-wine. They are known for their massive ability to fight cancer, and have also been implicated in the prevention of heart ailments to an appreciable extent. Sometimes, they serve as anti-inflammatory agents. Other phenolics include flavones, rutin, naringin, hesperidin and chlorogenic.

2.9.5 Saponins

The word ‘saponin’ is derived from *Saponaria vaccaria* (*Quillaja saponaria*), a saponin-rich plant, which was once used as soap. Saponins thus have ‘soap-like’ behaviour in water, i.e. they generate foam. Hydrolysis of saponins produces an aglycone, called sapogenin. Two types of sapogenin are steroid saponins and triterpenoid saponins. Generally, the sugar attachment is in C-3 in saponins. This is because there is a hydroxyl group at C-3 in many sapogenins. *Quillaja saponaria* contains

poisonous glycosides, quillajic acid and the sapogenin senegin. Quillajic acid is strenutatory and senegin is toxic. Senegin is also found in *Polygala senega*. Saponins are considered to be high molecular weight compounds where a sugar molecule is combined with triterpene or steroid aglycone. Saponins are known to be soluble in water but insoluble in ether. They are tremendously poisonous, because they cause blood heamolysis. They are equally known to cause poisoning in cattle (Kar, 2007). They have a bitter taste, and cause irritation in mucous membranes. They are usually shapeless in nature, soluble in alcohol and water, but never soluble in non-polar organic solvents such as benzene and n-hexane.

Saponins have important therapeutic activities because they have been reported to have hypolipidemic and anticancer activities. Saponins are also necessary for activity of cardiac glycosides. There are two steroidal sapogenins, diosgenin and hecogenin. Steroidal saponins are usually used in producing sex hormones for clinical use. Progesterone, for instance, is obtained from diosgenin. Diosgenin is the major starting material for the synthesis of progesterone. Sarker & Nahar (2007) opined that diosgenin is isolated from *Dioscorea* species, supplied previously from Mexico, and China presently. Other steroidal hormones, such as cortisone and hydrocortisone, are prepared using hecogenin as a starting material. This can be isolated from Sisal leaves which are abundantly found in East Africa (Sarker & Nahar, 2007).

2.9.6 Tannins

Tanins are extensively found in plants. Their molecular weight is generally high and they are phenolic compounds. Tannins are usually abundant in the roots, barks, stem as well as outer layer of plant tissues and are soluble in polar solvents, such as water and alcohol. One major feature of tannins is to 'tan' (turning things into leather) or precipitating gelatin from solution. This property

is usually known as astringency. Kar (2007) opined that reaction of tannins is acidic; and presence of phenolics or carboxylic group is accountable for this. With proteins, carbohydrates, gelatin and alkaloids, there is formation of complexes. There are two classes of tannins viz: hydrolysable tannins and condensed tannins.

There is production of gallic acid (usually as multiple esters with D-glucose) and ellagic acid when hyrolysable tannins undergo hydrolysis. Condensed tannins are more abundant (often called proanthocyanidins) and are obtained from flavonoid monomers (Cowan, 1999). Based on the acid produced, the hydrolysable tannins could either be gallotannins or egallitannins. When heated, pyrogallic acid is produced. Due to their phenolic group content, tannins are used as antiseptic. Hydrolysable tannins include daidezein, glycitein, theaflavins (from tea), and genistein. Medicinal plants that are rich in tannin are used as therapeutic agents for a couple of diseases. In their studies, Haslam (1996) and Stern, Hagerman, Steinberg, & Mason, (2006) revealed formation of complexes between tannins and microbial proteins at the molecular level. These complexes are formed through weak forces, such as hydrophobic effects, hydrogen bonding and covalent bonding. It is therefore possible that the mode of antimicrobial action of tannins could have a link with their capacity to inactivate enzymes, transport proteins, microbial adhesions, and cell membranes. Stern *et al*., (2006) suggested that tannins could equally act directly by inactivating microorganisms (eg. is the alteration of the morphology of germ tubes of *Crinipellis perniciosa* by low concentrations of tannin).

2.9.7 Terpenes

Terpenes are extensively distributed in natural products and are chemically diverse. They are generally found in essential oils, oleoresins or resins. These flammable unsaturated hydrocarbons

exist in liquid form (Firn, 2010). Terpenoids comprise hydrocarbons of plant origin that have the common formula $(C_5H_8)n$. They are classified as mono-, di-, tri- and sesquiterpenoids considering the number of carbon atoms. Some important monoterpenes include terpinen-4-ol, camphor, eugenol and menthol.

Diterpenes (C_{20}*)* include resins and taxol which are anticancer agents. The triterpenes (C_{30}) comprise sterols, steroids, and cardiac glycosides which have anti-inflammatory, sedative, insecticidal or cytotoxic properties. Examples of triterpenes include ursolic acid, amyrins, and oleanic acid. Martinez, Lazaro, Del Olmo & Benito, (2008) revealed that like monoterpenes, squiterpene*s* (C_{15}) are major components of many essential oils. Sesquiterpenes cause irritation when applied topically but when consumed, their action appears to be like gastrointestinal tract irritant. A good number of sesquiterpene lactones have been isolated and generally, they have antimicrobial and neurotoxic actions. A sesquiterpene lactone, palasonin, isolated from *Butea monosperma* exhibited antihelmintic activity. Terpenoids are grouped based on the number of isoprene units involved in forming of these compounds.

2.9.8 Steroids

Steroid glycosides (Plant steroids) or ‘cardiac glycosides; like other plant phytochemicals occur naturally. They are cardiac therapeutic agents (Firn, 2010). Cardiac glycosides are essentially steroids with a natural ability to exert powerful actions, especially on the cardiac muscle when administered via injection into animal or man. Madziga, Sanni & Sandabe, (2010) reported that anabolic steroids encouraged retention of nitrogen in osteoporosis. Steroidal glycosides should be used with caution because small amounts demonstrate the much needed stimulation on a diseased

heart. Death may result in excessive doses. Examples of plant steroids include diosgenin and cevadine (from *Veratrum veride*).

2.9.9 Anthraquinones

Anthraquinones are derived from phenolic and glycosidic compounds. They are exclusively obtained from anthracene, giving different oxidized derivatives, such as anthrones and anthranols (Maurya, Singh & Yadav, 2008 & Firn, 2010). The other derivatives, salinos poramide, aloe-emodin, rhein, chrysophanol, luteolin and emodin, have a common double hydroxylation position at C-1 and C-8. When a powdered plant material is mixed with organic solvent and filtered, and an aqueous base, e.g. NaOH or NH_4OH solution, is added to it, a pink or violet colour in the base layer shows presence of anthraquinones in the plant sample (Sarker & Nahar, 2007).

2.9.10 Essential oils

Essential oils comprise the aromatic, as well as volatile components of plants and animals. On exposure to air, essential oils evaporate even at ambient conditions. Consequently, they are said to be volatile oils or ethereal oils**.** Essential oils are secondary metabolites which are rich in compounds based on isoprene structure. Essential oils are responsible for the fragrance that emanates from most plants. They consist mainly of compounds that belong to the chemical group known as terpenes. When elements, such as oxygen, are present, they are referred to as terpenoids. Essential oils differ from fatty acids by their elaborate branching and cyclization, although terpenoids are synthesized from acetate units. Essential oils could either be secreted directly by the plant protoplasm or through hydrolysis of some glycosides. Some parts of the plant, known for secreting essential oils, include: Glandular hairs in Lamiaceae e.g. *Lavandula angustifolia*, Oil tubes (or vittae) (Apiaceae eg. *Foeniculum vulgare*, and modified parenchymal cells (Piperaceae

e.g. *Piper nigrum* - Black pepper. Essential oils can be obtained from leaves, stems, flowers, roots or rhizomes. One volatile oil may consist of more than 200 varying chemical components. The trace components are mainly accountable for the characteristic flavour and odour of leaves, stems etc as suggested by Firn (2010).

2.9.11 Antioxidants

Antioxidants are substances which prevent cells from being damaged by the effects of Reactive Oxygen Species (ROS) known as 'free radicals'. They have sufficient electrons which they easily donate to compounds or molecules that are electron-deficient. These electron-deficient compounds have unpaired valence electrons (Kadam, Joshi, Sawant & Jadhav, 2010; Aluko, Oloyede & Afolayan, 2013). In the bid to get more electrons, they may attack cells or biomolecules in the human body resulting in the generation of many diseases (Aluko *et al.*, 2013). As they get sufficient electrons, their actions are stabilized. The Reactive Oxygen Species (ROS) include peroxyl radicals, hydroxyl radicals, super oxide, and peroxynite whose presence leads to oxidative stress and consequently to cellular damage (Mattson & Cheng, 2006). The difference between antioxidants and Reactive Oxygen Species could result to oxidative stress and cellular damage. According to studies by Uddin, Akond, Mubassara & Yesmin, (2008) and Jayasri, Mathew & Radha, (2019), natural antioxidants serve the purpose of maintaining good health and well being, as well as prevention of chronic and degenerative diseases, such as cardiac and cerebral ischema, atherosclerosis, diabetic pregnancy, neurodegenerative disorders, carcinogenesis, rheumatic disorder, damage to DNA and ageing. Antioxidants act by searching out the free-oxygen radicals giving rise to stable radicals. The free radicals have the tendency to trap electrons from molecules in the immediate environment. If these radicals are not effectively scavenged and on time, they may cause severe damages to biomolecules, such as proteins, lipids, mitochondria and DNA; this

could lead to abnormalities and disease conditions (Uddin *et al*. 2008). Chen, Shieh, Kuo & Hsieh (2006) & Uddin *et al*. (2008) stated that free radicals are implicated in many diseases, such as hemorrhagic shock, atherosclerosis, tumour inflammation, diabetes, infertility, asthma, cardiovascular disorders, gastrointestinal ulcerogenesis, rheumatoid arthritis, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer's diseases), AIDS and early senescence. The human body does not produce enough antioxidants needed to prevent oxidative stress. Sen (1995) opined that free radicals produced in the body can be eliminated by the body's own natural antioxidant defences, such as glutathione or catalases**.** Several studies have reported that this deficiency can be remedied using natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, carotene and natural products in plants (Madsen & Bertelsen, 1995), Rice-Evans, Miller, & Paganga, (2017); Diplock, Charleux, Crozier-Willi, Kok, Rice-Evans, Roberfroid, & Vina-Ribes, (2018). Many free radical scavenging molecules, such as phenols, flavonoids, vitamins, terpenoids, which are rich in antioxidant activity, are naturally found in plants (Sun, 2003). Citrus fruits and green leafy vegetables, which contain vitamin E, carotenoids, ascorbic acid, flavanols and phenolics, exhibit free radical scavenging activities. In their studies, Hertog and Feskens (1993), Anderson and Teuber (2001) reported considerable antioxidant properties in phytochemicals which are necessary for reducing incidence of many diseases. Plant-derived polyphenolic compounds obtained from foods have proven to be more effective antioxidants *in vitro* compared to vitamins E or C. These can help appreciably in providing protective effects *in vivo* (Jayasri *et al.,* 2019). Antioxidants are usually added to foods as supplements to avoid the radical chain reactions of oxidation. These supplements act by interfering with the initiation and propagation step. This leads to termination of the reaction and delay in the oxidation process. As the concern for safety of synthetic compounds increases, food industries are now committed to

finding natural antioxidant sources to replace synthetic compounds. Some of the factors that can affect bioactive compounds in plants include: plant-part used, environment, seasons, intra-species variations, and age of plant. These parameters are perhaps accountable for the differences observed in *in vitro* and *in vivo* activities of plant parts used.

2.10 Extraction and Characterization Techniques

According to Handa (2008), extraction refers to the process by which substances in a plant material are separated using a particular solvent and standard procedure. Extraction remains the initial step in any medicinal plant study; it plays an important role in drawing conclusions from the study. Extraction methods are often referred to as sample preparation techniques. Several steps are involved in the extraction of plant materials. The first step is to reduce the size of dried plant materials. This aims at breaking the cell wall to ensure that all parts of the plant are exposed to the solvent. It also increases the surface area of plant materials and raises the transfer rate of soluble components from plant to solvent. The solvent extracts the soluble molecules and leaves the insoluble material behind. After extraction, with the desired solvent to get the desired components, filtration of the extract follows, in order to eliminate the bulk materials. The extract is then subjected to a concentration and drying process to remove the solvent and obtain a dry crude extract (Sarker, Latif & Gray, 2016).

Before extraction, the plant material is handled properly as it serves as the starting point of natural product isolation. Plant tissues are prepared such that the risk of contamination and losing valuable active compounds or the chemical constituents is minimized. In most cases, the plant tissue is dried; this is considered the best way to preserve plant materials. It is also suitable for long term storage of the samples (Harborne, 1998). The drying process is carried out in a controlled

condition to avoid altering the chemical constituents during the process (Seidel, 2016). The plant material is ground to improve the subsequent extraction. This process increases the surface area and facilitates penetration of solvents into the plant cells. Plant extract is made up of complex mixtures of substances. Accordingly, initial treatment is required to remove unwanted contaminants, biomass, etc before extraction. This equally prevents enzymatic oxidation or hydrolysis especially when extraction is done with fresh plant materials. It is carried out by immersing the plant materials in an organic solvent. The procedure and solvent used for extraction of a particular component from a plant is determined by the nature of the desired molecules. A non-polar solvent is used to extract a non-polar molecule while a polar solvent is used to extract a polar molecule. For thermolabile components, heat exposure is avoided by using cold extraction procedures. Nonpolar solvents are used to extract nonpolar substances, such as sterols, alkanes, pigments, fatty acids, waxes, coumarins and some terpenoids. On the other hand, polar solvents are used to extract polar compounds, such as glycosides, flavonoids, tannins, and alkaloids (Seidel, 2006).

However, regardless of the nature of the desired components, solvent characteristics are also considered. After extraction, solvent is easily removed from the extract via evaporation or distillation. As a result of this, solvents that can easily be recovered are preferred. Other factors to be considered include: physical and chemical properties of the solvents because they may affect the efficiency and effectiveness of the extraction procedure. Characteristic feature, such as solvent polarity, gives a hint of the characteristics of the active components responsible for the bioactivity of a particular plant. Table 2.4 shows solvents and their properties used for extraction of substances from plants materials.

Table 2.4: Solvents used for extraction of plant materials.

Solvent	Boiling Point	Dielectric constant	Dipole Moment	Polarity Index
Hexane	69°C	2.02	0	0.009
Diethyl ether	35°C	4.3	1.15	0.117
Ethyl acetate	77°C	6.02	1.78	0.228
Chloroform	61°C	4.81	1.04	0.259
Dichloromethane	40°C	9.1	1.6	0.309
Acetone	56°C	21	2.88	0.355
Acetonitrile	82°C	37.5	3.92	0.46
n-Butanol	118°C	18	1.63	0.602
Ethanol	79°C	24.55	1.69	0.654
Methanol	65°C	33	1.7	0.762
Water	100°C	80	1.85	1

Source: Seidel, (2006)

Common factors which affect extraction processes include matrix properties of the plant part, extraction time and solvents used. When the extraction process is concluded, further separation, identification and characterization of the bioactive compound becomes possible and easier. The bioactive compounds in a plant part can be extracted using several standard extraction methods. Most of these methods or techniques depend on the extracting power of the solvents used.

Several procedures are used for solvent extraction; they include infusion, decoction, maceration percolation and Soxhlet extraction. These procedures are based on the principle of solid-liquid extraction, because the plant material comes in contact with a particular solvent and allows diffusion of solvent into the plant cells. The solvent will then dissolve the substances in the plant cells. After extraction, the solvent is made to diffuse out of the cells (Seidel, 2006). Exposure to heat and evaporation should be avoided because they cause loss or degradation of the constituents of the plant. Hence, the extraction procedure has to be carefully considered.

Plant extract is made up of complex molecules with different biological, chemical, and physical properties. This makes it difficult to obtain pure molecules using one isolation procedure. Different isolation procedures are usually employed to isolate single compounds. Choice of these procedures depends on the nature of the compound to be isolated. One of the most powerful techniques used in the isolation and purification of natural products is chromatography.

2.11 *In Vitro* and *In Vivo* Antimalarial Activities of Medicinal Plants Used in the Treatment of Malaria

Problem posed by malaria scourge has necessitated several research works to be carried out, especially in the tropics where malaria transmission is very high. These studies involve both *in vitro* and *in vivo* activities of medicinal plants used traditionally for treatment of malaria. These medicinal plants are evaluated, investigated, studied, analyzed etc for antimalarial activities; this is one of the strategies adopted by scientists in malaria endemic regions of the world to authenticate, rationalize or support use of such medicinal plants as malaria therapy. According to Anderson (2007), these vast research works also aim to produce the next inexpensive, effective, affordable and safe antimalarial drugs.

In *in vitro*, antiplasmodial studies are used for assessing response of fresh isolates of *Plasmodium* species obtained from malaria patients (Basco & Ringwald, 2007) or from continuous cultures of chloroquine-sensitive or-resistant strains of *P. falciparum* (Wan-Omar, 2007). Alternatively, evaluation of *in vivo* antiplasmodial activity involves inoculating experimental animals, such as rats and mice with *Plamodium berghei.* Drugs or extracts are then administered in order to evaluate the therapeutic effects of such extracts (Ogbunufagor, Okochi, Okpuzor & Emeka, 2008). Many Nigerian medicinal plants have demonstrated fascinating potentials as antimalarial agents for taking care of various strains of resistant malarial parasites. These *Plasmodium* species (causative agents for malaria) have constantly proven resistant to many approved antimalarial drugs, making malaria control a difficult task.

Efficacy of crude organic extracts from four medicinal plants widely used in Nigeria by local healers against fever and malaria was tested *in vitro* against *P. falciparum* (Gill & Akinwummi, 1996). Findings showed that extract obtained from the plant Oliver (*Enathia chlorantha*), was very active; high percentage inhibition was recorded against the parasites for all the concentrations used for the study. Another study by Awe & Makinde (1997) revealed that extracts of the plant *Morinda lucida* showed dose-dependent inhibitory results. They reported dose-dependent and seasonal variation in the antimalarial activity of this plant by using both *in vitro* and *in vivo* methods. The phytochemicals contained in this plant (*M. lucida*) included anthraquinones which demonstrated *in vitro* activity against *P. falciparum.* It also had antifungal activity. *Morinda lucida* is usually used locally for treatment of yellow fever as well as jaundice (Guido, Menavanza, & Kurt, (2015). Weenen (1990), reported that organic leaf extract of *Vernonia lasiopus* demonstrated high *in vitro* inhibition against malaria parasites, with IC_{50} value of 1.0 μg/ml. Medicinal plants, *Sarcocephalus latifolius*, *Alstonia boonei*, *Petivera alliacea*, *Mangifera indica* and *Khaya grandifolia* also

exhibited significant antimalarial properties (Awe, Olajide, Oladiran, Makinde, 2008; Guede, Lengo, Frederic, Bernard & Philippe, 2015). In another study on the *in vitro* antimalarial activity of extracts of *Vernonia amygdalina*, commonly used in traditional medicine in Nigeria, Sha'a, Oguche, Watila & Ikpa, (2011) reported significant inhibition in schizont maturation relative to the control. Ethanolic extract of the plant exhibited higher antimalarial activity of 78.10 % and IC_{50} of 11.2 µg/ml while the aqueous extract exhibited an activity of 74.02 % and IC_{50} of 13.6 µg/ml. The two extracts exhibited reasonable antimalarial activity. The extracts were also noted to be non toxic in rats; they equally recorded good anti-inflammatory activity. This explains why the plant is used traditionally for treatment of malaria. Omorejie & Sisodia (2012) investigated *in vitro* antiplasmodial activity and cytotoxicity effect of leaf extracts of *Jatropha tanjarensis*. The ethanolic extracts of the plant had very high antiplasmodial activity (IC_{50} 10.86±1.52 µg/ml), low cytotoxicity (IC_{50} 86.8±4.8 µg/ml) and selectivity index (S.I) of 8.0. Results obtained authenticated the traditional claims for the use of this plant in the treatment of malaria. Olasehinde, Anyanda & Nwabueze (2012) studied *in vitro* antiplasmodial activity of n-hexane and ethanolic extracts of *Moringa oleifera* seeds on *Plasmodium berghei*. The ethanolic extracts recorded 61% parasite inhibition at a concentration of 50 mg/kg, 65% at 100 mg/kg and 100% at 200 mg/kg concentration after a 72-hour period of treatment. *In vitro* antimalarial activity of aqueous and ethanolic extracts of *Anacardium occidentale* against *Plasmodium falciparum* in Damboa, North Eastern Nigeria was carried out by Sha'a, Ajayi & Arong, (2014). The ethanolic extract of the plant showed a higher *in vitro* antimalarial activity of 75.64%, IC_{50} of 11.7 µg/ml while the aqueous extract showed antimalarial activity of 72.38%, IC_{50} of 16.00 µg/ml. Appiah-Opong (2011) evaluated the antiplasmodial activity of extracts of *Tridax procumben*s and *Phyllanthus amarus* in *in vitro Plasmodium falciparum* culture systems. Results obtained from this study showed antiplasmodial

activities against chloroquine resistant *Plasmodium falciparum*. IC_{50} values were 24.8 µg/ml for *Tridax procumbens* and11.7 µg/ml for *Phyllanthus amarus*. *In vitro* antimalarial effect of combined extracts of the leaf of *Ficus exasperata* and stem bark of *Anthocleistha vogelli* on mice experimentally infected with *P.berghei Berghei* (NK65) was studied by Okon, Gboeloh & Udoh, (2014). Results obtained revealed dose-dependent chemosupressive activitities of 68.8, 79.5 and 91.7%, corresponding to dose levels of 100, 200 and 400 $mgkg^{-1}$ b.wt. There was also increase in size of liver and spleen in the infected mice compared to uninfected ones. Combined extracts showed acute toxicity (LD_{50}) of 3162 $mgkg^{-1}$ b.wt. This result justifies the traditional use of these plants in malaria treatment.

Melariri, Campbell, Etusim, & Smith, (2012) studied *in vitro* antiplasmodial activities of extracts from five plants used singly and then in combination against *P. falciparum* parasites. Findings from this study revealed that using the extracts singly showed activity against strains of malaria parasites. Nevertheless, when the extracts were combined (synergism), activity of the extracts was increased, suggesting that resistance of parasites can be tackled with a combination of antimalarials. *In vitro* antiplasmodial activity and phytochemical screening of *Newbouldia laevis* used in treating malaria symptoms was carried out by Enemakwu, Adeyemi & Salihu, (2016). They reported that percentage of parasites removed increased as concentration of extracts and fractions increased. *In vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine by Abiodun, Gbotosho, Ajaiyeoba, Happi, Falade, & Oduola, (2011) revealed that the plant extracts were active against malaria parasites and could be used in the management of malaria. *In vitro* antiplasmodial and cytotoxic activities of plants used as antimalarial agents (*Cassia siamea* stem bark, *Tithonia diversifolia* leaf, and *Cajanus cajan* leaf) in Southwest Nigeria ethnomedicine were studied by Ajaiyeoba, Oladepo, Fawole, Bolaji, Akinboye,

Ogundahunsi, Falade, Gbotosho, Itiola, Happi, Ebong, Ononimu, Osowole, Oduola, Ashidi & Oduola, (2005). Antiplasmodial activity of 24.9 µg/ml, 52.9 µg/ml and 53.5 µg/ml from methanolic extracts of these plants were reported. *In vitro* antiplasmodial activities of some Central American medicinal plants were tested (Jenett-Siems, Mockenhaupt, Bienzle, Gupta & Eich, 2019). The authors concluded that selecting plants by ethnobotanical criteria may provide potential sources for new antimalarial compounds. *In vitro* antimalarial activities of the medicinal plants, *Swartzia madascariensis, Cumbretum glutunosum* and *Tinospora bakis* from Burkina Faso were evaluated by Ouattara, Sanon, Traore, Mahiou, Azas & Sawadogo, (2016). Results obtained showed that extracts of *S. madagascariensis, C. glutunosum* and *T. bakis* have antimalarial activity.

In vitro antiplasmodial activity of the plant, *E. littorale* against *P. falciparum*, and potential antimalarial action of the plant has been reported by Soni & Gupta (2009). Ngemenya, Akam, Yong, Tane, Fanso-Free, Berzins & Titanji, (2016) also analyzed *in vitro* activities of extracts of *Turreanthus africanus*, a medicinal plant used in Southwest Cameroon for treating malaria. Their studies revealed that *T. africanus* did not have strong antiplasmodial activity; they opined that the activity may be enhanced when the plant is combined with other antiplasmodial plants (synergism). *In vitro* antimalarial activities of quinones from *O. basilicum*, *Garcinia kola*, *Cassia alata* and *C. occidentalis* (Kayembe, Taba, Ntumba, Tshiongo & Kazadi, 2010) and activity of crude extracts of 12 popular plants in Benin for treatment of malaria (Bero, Ganfon, Jonville, Fredrich, Gbaguidi, DeMol & Quetin-Leclercq, 2019) recorded antimalarial activities by the plants. Mamadou, Kirkman, Diarra, Goita, Doumbia, Traore & Diallo, (2017) studied *in vitro* antiplasmodial potentials and phytochemical screening of ten plants popularly used as

antimalarials in Mali; the aqueous and ethanolic extracts of the selected plants were active against *Plasmodium falciparum*.

According to Peters (2005), *in vivo* antimalarial and cytotoxic studies on extracts from *Annona senegalensis* (a famous plant used by traditional medicine practitioners) showed some inherent antimalarial properties. This was revealed based on the percentage of chemosuppression recorded when compared to chloroquine in a 4-day suppressive test. This study showed that *Plasmodium berghei*-infected mice treated with methanolic extracts of *A. senegalensis* recorded a dose-dependent chemosuppression when compared to chloroquine-treated controls. Results obtained showed that the group of mice treated with 800mg/kg recorded the highest percentage of chemosuppression. Alkaloids present in this plant were accountable for the antimalarial activity. Alkaloids are reported as a key bioactive agent in *Annona* species (Gbeasor, Kedjagni, Koumaglo, de Souza, Agbo, Aklikokou & Amegbo, 2019; Ruprrecht, Hui & McLaughlin, (2019).

In their report, Okpaniyi & Ezeuku (2011), opined that the comparatively lower inhibition recorded in their *in vivo* antimalarial study of organic extracts of *Azadirachta indica* supports earlier findings that *A. indica* acts as an antipyretic than as a schizonticidal agent in the treatment of malaria. Edith, Folade, Ogbole, Okpako & Akinboye, (2016) reported that effects of boiled water extracts of *Cymbopogon giganteus* and *Enantia chlorantha* on chloroquine - resistant *Plasmodium yoelii nigeriensis* in albino mice for antimalarial activity showed that extracts from these two plants took care of the infection in a dose-dependent manner. Similar studies on effects of another four Nigerian medicinal plants against chloroquine-resistant *P.yoelii nigeriensis* in mice revealed a high chemosuppressive and prophylactic activity (Kimbi, Fagbenro-Beyioku & Oyibo 2018). The study also showed that boiled water extracts from *C. giganteus* and *E.chlorantha* were

active against chloroquine-resistant *P.yoeli nigeriensis*, either as schizonticidal or prophylactic agents when compared to artemether. Makinde & Obih (2005) reported very little antimalarial activity for extracts of *A. indica* and *M. lucida* in mice. According to them, there was schizonticidal activity against chloroquine-sensitive *P.berghei* when the boiled water extract of *A. indica* was used. Strains or species of parasites used were held accountable for this difference. Kimbi *et al*., (2018) & Krettli (2001) opined that there is possibility that several plants used for treating fever or malaria in some areas could be inactive or toxic to mice; animal model used for *in vivo* assessment may not be appropriate to exhibit the desired antimalarial activity. In their study on bioguided investigation of the antimalarial activities of *Trema orientalis* (L.) blume leaves, Babatunde *et al*. (2015) reported that when graded doses (100 to 800 mg/kg) of acetone extract of *T. orientalis* were used in treating *Plasmodium berghei* infected mice, there was significant chemosuppression of parasite growth, ranging from 44.0 to 83.8%. Ethanolic leaf extract of the plant, *Pseudocedrala kotschyi,* recorded antimalarial potentials; the leaf extract of the plant (100-400 mg/kg b.wt) reported significant dose-dependent activity against the plasmodium in the suppressive and curative tests Akuodor, Ajoku, Ezeunala, Chilaka & Asika, (2015). The result also revealed that the ethanolic leaf extract prolonged survival period of the infected mice. LD_{50} of the plant extract was recorded as ≥ 500 mg/kg b.w. in mice. This work justified that the plant has antiplasmodial activity, thus can be used for malarial treatment. Sanganuwan, Onyeyili, Ameh & Etuk, (2011) studied *in vivo* antiplasmodial activity of aqueous extracts of *Abrus precatorius* in mice. The extract had antiplasmodial activity on *P. berghei,* justifying use of this medicinal plant against malarial parasites. Adebayo, Odediran, Aliyu, Nwafor, Nwoko & Umana, (2014) evaluated *in vivo* antiplasmodial potentials of a combination of four Nigeria antimalarial plants. A combination of these medicinal plants was more effective than when used singly, further justifying

that a combination of different medicinal plants (synergism) could be more effective in treating malaria. Ior, Wannang, Ior & Amagon, (2015) in their study on *in vivo* assessment of the antimalarial activity of *Cassia singueana* and *Cymbopogon citratus* reported that the extract exhibited significant chemosuppresive effect of from 72.7% to 90.5% from *Cassia singueana* (400-600 mg/kg). Prophylactic effect against *P. berghei* was between 79% and 83%; chemosuppressive effect of 20.83% to 80.56% and 55.38 to 78% from leaf and root extracts of *Cymbopogon citratus*. This is an indication that the plants are efficacious in treatment of malaria. *In vivo* antiplasmodial activities of *Russelia equisetiformis* in *Plasmodium berghei* infected mice conducted by Ojurongbe, Olasehinde, Akinjogunla, Egwari & Adeyeba, (2015) revealed a dose-dependent activity (100, 200, 400 and 800 mg/kg) in the chemosuppresion of *P.berghei* parasites by 31.6, 44.7, 48.4 and 86.5% respectively. No sign of toxicity was recorded in the extract even with the highest dose (5000 mg/kg). *In vivo* activity of hydroethanolic stem extract of *Baphia pubescens* on *P. berghei* infected albino mice was carried out by Ihekwereme, Agbata, Agbata Chukwueze & Agu, (2016). There was dose-dependent curative activity (100,200, and 400 mg/kg); 400 mg/kg dose recorded 100% parasite clearance after a 4-day treatment period. The extract was also noted to be non-toxic.

Fentahun, Makonnen, Awas & Giday, (2017) similarly studied *in vivo* antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice. Their findings showed that all the crude extracts were not toxic to the mice themselves, even with the highest dose (200 mg/kg). The solvent fractions of the plant were significantly inhibitory to the parasites. The highest dose (600 mg/kg) of aqueous and hydro-methanolic extracts recorded 95.5 and 93.7% parasite suppression. This result thus provides support for the local therapeutic use of *Strychnos mitis* in treatment of malaria.

Okokon, Antia, Mohanakrishan & Sahal, (2017) reported that husk extract of *Zea mays* (187-748 mg/kg b.wt) with LD_{50} of 1874.83 mg/kg exerted significant *in vivo* antimalarial activity against *Plasmodium berghei* in suppressive, prophylactic and curative tests. The ethyl acetate fraction exerted the highest activity with IC_{50} values of 9.31±0.46 µg/ml for chloroquine sensitive (Pf 3D7) and 3.69±0.66 µg/ml for chloroquine resistant (Pf INDO) strains of *P. berghei*. The crude extract equally showed no toxic effect on the two cell lines tested. In a related study by Ajayi & Adeyemi, (2017), on *in vivo* antiplasmodial activities of ethanol extracts of *Euphorbia hirta* whole plant and *Vernonia amygdalina* leaves in *P. berghei* infected mice, results obtained showed that whole plant of *Euphorbia hirta* and leaves of *Vernonia amygdalina* mildly inhibited *P.berghei* schizont maturation by 44.36 and 37.85% respectively. Extracts of *Zanthoxylum gilletii* had significant antiplasmodial activity against chloroquine- resistant, chloroquine-sensitive strains of *P. falciparum* with IC_{50} values of 2.52, 1.48 and 1.43 µg/ml respectively (Omosa & Okenwa 2017). Another study by Innocent, Moshi, Masimba, Mbwambo, Kapingu & Kamuhabwa, (2009) on locally used plants in Tanzania for *in vivo* antimalarial activity in mice reported that the extracts of six plants used locally as medicine showed *in vivo* antimalarial activity, while three had no strong activity against malaria parasites. Therefore, they concluded that the roots of *Caesalpinia bonducella* and ethanol extracts of the leaves of *Cassia abbreviate* have promising potentials. *In vivo* anti malarial effects of methanol and aqueous extracts of different parts of *Picralima nitida* by Ene, Obika, Okwu, Alisi & Edeh, (2013) revealed that methanol extracts of the root and stem bark of this plant showed higher antimalarial activity when compared to other extracts. Mgbemena, Opara, Ukaoma, Ofodu, Njoku & Ogbuagu, (2010) studied *in vivo* prophylactic potential of lemon grass and Neem as antimalarial agents. They reported antiplasmodial activities in both plants.

2.12 Use of *Plasmodium berghei* in Antimalarial Studies

Plasmodium berghei is a protozoan parasite which causes malaria in rodents. It was originally isolated from thicket rats in Central Africa; it is one of the four species described in African murine rodents; others are *chabaudi*, *vinckei*, and *yoelii*. Because of its ability to infect rodents and relative ease of being genetically engineered, *P. berghei* is a popular model organism for the study of human malaria. The parasite is found in the forests of Central Africa, where its natural hosts are the thicket rat (*Grammomys surdaster*) and the mosquito (*Anopheles dureni*). *P. berghei* was first identified in the thicket rat (*Grammomys surdaster*). In research laboratories, various rodents, such as mice, rats, can be infected (Junaid, Khaw, Mahmud, Ong-Kien, Lau, Borade, Liew, Sivanandam, Wong & Vythilingam, 2017). The natural insect host of *P. berghei* is likely *Anopheles dureni*; however in laboratory conditions it has also been reported to infect *Anopheles stephensi*.

Like the other human malaria parasites, *P. berghei* is transmitted by *Anopheles* mosquitoes and it infects the liver after being injected into the bloodstream through the bite of an infected female mosquito. Few days after the bite, development and multiplication, these parasites leave the liver and attack the erythrocytes (red blood cells). Multiplication of the parasites in the blood results in pathology, such as anaemia or damage of essential organs of the host, such as liver, lungs, and spleen. *P. berghei* infections could also lead to brain damage and cerebral complications in laboratory mice. These symptoms have been reported to be comparable to symptoms of cerebral malaria in patients infected with the human malaria parasite, *Plasmodium falciparum* (Franke-Fayard, 2010).

P. berghei infection of laboratory mouse strains is usually used in research as a model organism for human malaria (Craig, Grau, Janse, Kazura, Milner, Barnwell, Turner & Langhorne 2012). A number of commercially available laboratory mouse strains have been used to replace the normal host. *P. berghei* is used as a model organism for the investigation of human malaria due to its similarity to the *Plasmodium* species which cause human malaria. *P. berghei* has a very similar lifecycle with the species that infect humans. It also causes disease in mice and this has similar symptoms with those seen in human malaria. Importantly, *P. berghei* is easily genetically manipulated more than the species that infect humans, making it a useful model for research into *Plasmodium* genetics. This parasite is equally used for the analysis of the function of malaria genes using the technology of genetic modification (Janse, Ramesar & Waters 2011; Khan, Kroeze, Franke-Fayard & Janse, 2013).

Furthermore, the genome of *P. berghei* has been sequenced and it shows a high similarity, both in structure and gene content, with that of the human malaria parasite, *Plasmodium falciparum* (Hall, 2005; Kooij, Janse & Waters, 2014; Otto, 2014). *P. berghei* is also used in research programs for development and screening of anti-malarial drugs and for the development of an effective vaccine against malaria (Khan, Janse, Kappe & Mikolajczak, 2012).

However, in somes aspects, the pathology caused by *P. berghei* in mice is different from malaria caused by *P. falciparum* in humans. Death resulting from *P. falciparum* malaria in man is often caused by the accumulation of red blood cells in the blood vessels of the brain but in infection due to *P. berghei* in mice are found to have an accumulation of immune cells in brain blood vessels (Craig, Grau, Janse, Kazura, Milner, Barnwell, Turner & Langhorne, 2012).

2.13 Acute Toxicity Test

Acute toxicity test is the first step employed during exploration of an unidentified substance. The median lethal dose (LD_{50}) is the index of acute toxicity. This is the lethal dose which can kill 50% of experimental animals. The need for acute toxicity studies need not be overemphasized. In as much as the scientific study of most medicinal plants has verified and authenticated use of these plants in managing several diseases, herbal mixtures remain a composition of several bioactive phytochemicals that may have different mechanisms of action (Sengupta, Sharma & Chakraboty 2011). Different levels of toxicity may occur from the potent toxic compounds present in these medicinal plants. Non-toxic compounds may also behave like toxic compounds, even at lower doses and can produce undesirable effects by interacting with human or animal cells. Therefore, some medicinal plants may not be safe for consumption in the crude form. Such plants should be properly investigated for a better understanding of their properties, safety and efficiency. Acute toxicity test is therefore a necessary step for any therapeutic agent proposed for human use. CDER (1996) affirmed that findings from these studies help in choosing doses for repeat-doses studies.

Tedong, Dzeufiet, Dimo, Asongalem, Sokeng, Flejou & Kamtchouing, (2017) carried out acute toxicity and subchronic toxicity tests of *Anacadium occidentale* leaves. After administering the extracts orally, they reported no toxicity in doses of extract < 6 g/kg body weight. LD_{50} of the extract, in male and female mice after oral administration was 6g/kg. They therefore concluded that at increased doses greater than those employed in the models of antidiabetic activities, toxic effects of *A. occidentale* hexane extract may occur. A similar study on the effect of ethanolic extract of cashew (*A.occidentale*) stem bark on the brain and kidney of Swiss albino mice by Ofusori, Enaibe, Adelakun, Adesanya, Ude, Oluyemi, Okwuonu, and Apantaku (2008), recorded non-toxic effect of ethanol extract of *A.occidentale* on the brain and kidney parenchyma of the

mice. Idowu, Okoronkwo and Adagunodo, (2009) reported that in ethnobotanical survey of antimalarial plants in Ogun State, Southwest Nigeria, administration of *C. citratus* was not toxic. Oral administration of the extract of *Cassia fistula* at the highest dose of 5000mg/kg on mice recorded no sign of mortality (Subramanion, Zuraini, Yeng, Yee, Lachimanan & Sreenivasan, 2011). There was no adverse effect and biochemical analysis did not reveal any significant elevation in all the parameters, indicating that *C. fistula* is not toxic and can be safely used in pharmaceutical formulations.

A study on the acute and sub-acute toxicity effect of *Senna alata* in swiss albino mice revealed that the highest dose administered (3000 mg/kg b.wt) recorded no mortality nor changes in the general behavior of the experimental animals (Roy, Ukil & Lyndem, 2016). The studied parameters remained unaltered. Akinpelu, Apata, Iwalewa & Oyedapo (2016) reported that ethanolic leaf extract of *Clerodendrum volubile* contained bioactive compounds with hypolipidermic effect that were more active as curative agents than prophylactic agents. They attributed this to the plant's high phenolic and flavonoid content of the plant. It thus justifies use of the plant in treatment of hyperlipidermia-related diseases. Similarly, Ali, Fatina & Bashir, (2017) carried out a study on the protective effects of crude and alkaloidal extracts of *Temarindus indica* against acute inflammation and nociception in rats. The safe dose and LD_{50} for crude methanolic extract were 400 and 750 mg/kg respectively while those of the alkaloidal extract were 40 and 57 mg/kg respectively. The plant is thus used in folk medicine. Nda-Umar, Gbate, Alfa & Mann, (2017) also reported that extracts of *L.bateri* and *P.thonningii* leaves recorded an oral LD_{50} of > 2500 mg/kg b.wt, *C. aculeatum* showed LD50 of > 1500 mg/kg b.wt while *C.adansonii* root recorded LD50 of > 2000 mg/kg b.wt in mice. Prasad & Venugopal (2016) reported that extract of *Argemone mexicana* root did not record any evident clinical signs of toxicity and mortality during a 14-day test period, even

with the highest dose of 5000 mg/kg b.wt. The parameters studied were not significantly different compared to the control group. Lalitha, Sripathi & Jayanthi, (2012) also studied acute toxicity of extracts of *Eichhornia crassipes* (Mart). Results obtained showed no mortality and there were no changes in the behavior of the experimental animals, even with the highest administered dose of 2000 mg/kg body weight, indicating that the plant extract was safe for consumption. An LD_{50} of greater than 5000 mg/kg body weight was recorded when aqueous extract of *T. avicenniodes* was administered on white albino rats (Bulus, Atawodi & Mamman, 2011). Liver congestion was recorded in 100 mg/kg body weight dose group. Safety of this plant in traditional medicine was upheld.

Acute and sub-acute toxicity studies on aqueous and methanolic extracts of *Nelsonia campestris* in rats were carried out (Olaniyan, Muhammad, Makun, Busari & Abdullahi, 2016). A moderate hepatic and cortical necrosis of liver and kidney, when the two extracts were administered at 100 and 600 mg/kg body weight, was recorded. Lymphatic infiltration and portal congestion were also reported when doses of 300 mg/kg body weight of the aqueous and methanolic extracts were given. High doses of the extract of this plant could thus lead to mild organ toxicity.

A probability of hepatotoxic effect of the root extract of a plant, *Euphorbia lateriflora* after administering 100 mg/kg and 250 mg/kg body weights was recorded in rats (Usman, Sule & Gwarzo, 2014). They reported likely kidney malfunction, oral LD_{50} above 5000 mg/kg, and histopathological lesions on the liver and kidney after acute toxicity study.

No significant changes were reported when leaf extracts of *Carica papaya* were administered in rats (Halim *et al*., 2011); and no abnormal signs were also recorded in rats treated with methanolic of extract of *Ceiba petandra* (Ghandare, Kavimani & Rajkapoor, 2013).

CHAPTER III: MATERIALS AND METHODS

3.1 Reagents and Chemicals Used

Methanol (JHD chemical) China, Ethanol (Analar) China, Hexane (Analar), Diethyl ether (Analar), Chloroform (Analar), Petroleum ether (Analar), Fehling's solution A and B, sodium hydroxide (NaOH), distilled water, Wagner's reagent, Dregendroft's reagent, Meyer's reagent, lead acetate, HCl, $FeCl_2$, Acetic anhydride, NH_2OH, Dinitrosallcyclic acid (DNS), iron III chloride, ammonium thiocyanate, Libermann-Burchard reagent, Giemsa stain, Silica gel (70-230 mesh).

3.2 Equipment and Instruments Used

Dry oven (DHG-9101-SA) Search Tech Instrument UK, Rotary evaporator (RE- 52A, Searchtech Instrument), water Bath (TT-6)Techmel & Techmel USA, Electro-thermal incubator DNP Search Tech Instruments China, UV-VIS Spectrophotometer Spectrum Lab 7555 UK, S. Mettler analytical weighing machine FA2104 UK, Chromatographic column, Gas chromatograph (BUCK M910), FTIR spectrophotometer (Agilent Cary 630), Gas chromatograph (5890-11) SHIMAZU, Japan, Centrifuge 80-2 Search Tech Instrument UK, De-Ionizer 50 Search Tech Instrument UK, electric blender (Kenwood-BL440A-UK), standard test sieve ASTM Search Tech Instrument UK, Test tube (Pyrex), Petric dish (Pyrex), Glass spreader, Bunsen burner, pipette and pipette filler, conical flask, refrigerator, spatula, slides & cover lips, microscope (B-Bran) Search Tech Instrument UK.

3.3 Collection of Plant Materials

Fresh leaves, stems and roots of *Chasmanthera dependens* and *Dictyandra arborescens* were collected from Ahiazu Mbaise L.G.A of Imo State, located between latitude 5°19' and 5°32'N and longitude 7°12' and 7°20'E (Ume, Jiwuba, Obi & Dauda, 2016). They were verified and identified by a plant taxonomist in the Department of Biological Sciences, Federal University of Technology, Owerri.

3.4 Preparation of Samples

The fresh leaves, stems and roots of *Chasmanthera dependens* and *Dictyandra arborescens* were sorted and rinsed with clean water, and air-dried at room temperature (30 ± 0.5^0C) for 2 weeks to a constant weight. This was done to avoid possible degeneration and possible loss of the bioactive compounds. The dried leaves, stems and roots were pulverized to coarse powder using electric blender (Kenwood-BL440A-UK). The coarse powder was then sieved, using a 2 mm mesh sieve, to obtain smooth samples.

3.5 Aqueous Extraction

Two hundred grams (200 g) of each plant part was mixed separately in 1000 ml of distilled water, using the cold maceration method (Ene *et al*., 2013). The samples were macerated for a period of fourty eight hours. This was done to make water accessible to the various phytochemicals present in the plant parts. The samples were then filtered, using a clean muslin cloth, then with whatman number 1 filter paper to remove residues. The filtrates were concentrated and dried using a rotary evaporator (RE- 52A, Searchtech Instruments) set at a temperature of 50°C to remove the solvent. The roots, stem and leaves of the two plants under study (*Chasmanthera dependens and*

Dictyandra arborescens) were extracted with water to give six (6) aqueous extracts. These extracts were then stored in well-labeled bijou bottles and kept in the refrigerator at 4ºC for subsequent use.

3.6 Methanolic Extraction

This extraction was done according to Dejen, Assefa, Teshome and Engidawork, (2018). Two hundred grams (200 g) of various parts of the two study plants were extracted with 1000 ml of 80% v/v methanol solvent using soxhlet extraction. The samples were first of all wrapped in a thimble and placed in the soxhlet reflux flask. The flask was mounted on an extraction flask containing 1000 ml of methanol. The upper end was connected to a condenser with all the connection points air-tight. The solvent was heated through an electro-thermal heater until it boiled. It condensed in the reflux flask until the wrapped samples were completely immersed in the solvent. The samples remained in contact with the solvent until the flask was filled and siphoned over, carrying extracts back to the boiling flask. This extraction was allowed for six (6) hours to ensure maximum extraction. The filtrates were equally evaporated to dryness using a rotary evaporator (RE- 52A, Searchtech Instruments). The roots, stems and leaves of the two plants under study (*Chasmanthera dependens and Dictyandra arborescens*) were also extracted with methanol to give six (6) methanolic extracts. These were also stored in properly labeled bijou bottles kept in the refrigerator at 4ºC for further use.

3.7 Experimental Animals

Only male Swiss albino mice, weighing 18-25g (7 to 8weeks), were used in this study; they were obtained from animal house of Department of Veterinary medicine, University of Nigeria, Nsukka and transferred to animal house of the Department of Biochemistry, University of Nigeria, Nsukka, where the study was carried out. These animals were acclamatized for 14 days with free access to

feed (rat pellets) and water *ad libitum* and monitored under 12 hours, light and dark cycles, in well aerated cages. They were then separated into their respective groups according to their body weights.

3.8 Acquisition of *Plasmodium berghei*

Chloroquine-resistant malaria parasite (*Plasmodium berghei* NK65) used in this study was obtained from the parasitology unit of Department of Veterinary Medicine, University of Nigeria, Nsukka. The *P.berghei* was maintained by sub-passaging into apparently healthy mice every five days throughout the duration of study.

3.9 Acquisition of Standard Antimalarial Drug- Artesunate

Artesunate tablets (Jawa Pharmaceuticals) were obtained from Milan Pharmacy Limited, Owerri, Imo State.

3.10 Qualitative and Quantitative Determination of Phytochemicals

Qualitative and quantitative phytochemical studies were carried out on the extracts to determine the bioactive components present in the plants' extracts.

Qualitative determination of alkaloids, steroids, terpenoids, glycosides, oxalate, flavonoids, tannins, phytate, saponins, and phenols was carried out as described by Harbourne, Boulter & Turner, (1998) and Ejikeme, Ezeonu & Eboatu, (2014).

3.10.1 Tannins

Phlobotannins react with dilute HCl to give a red colour precipitate under acidic conditions. 3.0 ml of extract was added to 2.0 ml of 1% HCl. Presence of red precipitate indicated presence of phlobotannins.

Follins-Dennis titrating method, as described by Uyotismita and Arindan (2015), was used for quantitative determination of tannins. Five grams of powdered sample was added to 100 ml of petroleum ether in a conical flask and left for 24 hours. This was then filtered and allowed to stand for 15 min. so that the petroleum ether will evaporate. The mixture was re-extracted for 4 hours by soaking in 100ml of acetic acid in ethanol. The solution was then filtered and the filtrate was collected.

To the filtrate obtained, 25 ml of NH_4OH was added to precipitate the alkaloids. Some of the NH_4OH in solution were removed by heating the alkaloid on a hot plate. 5 ml was taken from the remaining volume and 20 ml of ethanol was added to it. This was then titrated with 0.1M Sodium hydroxide (NaOH), using phenolphthalein as an indicator, until a pink colour was observed. This indicated the end point.

The quantity of tannin was then calculated in percentage (%) thus:

($C_IV_I = C_2 V_2$) molarity

Calculation:

Tannic acid, $C_IV_I = C_2 V_2$

Where C_1=concentration of Tannic acid

C_2=concentration of Base = 0.1 M

V_1= volume of Tannic acid = 5ml

V_2= Titre value of sample

Tannic acid $C_1 = \frac{C2V2}{V1}$

$$\% \text{ of tannic acid content} = \frac{C1 \text{ X } 100}{\text{weight of sample analysed}}$$

3.10.2 Saponins

(a) **Frothing test:** Two grams of sample was boiled with 20 ml of distilled water and filtered. 10ml of the filterate was added to 5 ml of distilled water and shaken vigorously until a persistent froth appeared. Three drops of olive oil were added and shaken vigourously until it formed an emulsion which confirmed presence of saponins.

The quantity of saponin in each sample was determined by adding 5 g of the sample into 20% acetic acid and ethanol; it was allowed to stand in a water bath at 50ºC for 24h. Extract from the filtered solution was concentrated to about one-quarter of the original volume on a water bath. Concentrated Ammonium hydroxide (NH_4OH) was added to the extract dropwise until a precipitate was formed. The solution was kept to settle and the precipitate was collected by filtration and weighed. The saponin content was thereafter calculated in percentage (Harbourne *et al.,* 1998).

Calculation:

$$\% \text{ saponin} = \frac{(\text{weight of beaker+residue})-(\text{weight of filter paper})\text{x}100}{\textit{weight of sample analysed}}$$

3.10.3 Flavonoids

Sodium Hydroxide test

1ml of dilute sodium hydroxide solution was added to 1 ml of extract in a test tube and the mixture was observed for any change in colour. Presence of flavonoids was indicated by formation of yellow precipitate (Ejikeme *et al*., 2014).

For quantitative determination, five grams of each plant sample was extracted with 100 ml of 80% aqueous methanol at room temperature in a conical flask. The solution was filtered and the filtrate

evaporated to dryness, using a water bath. The sample was then weighed until a constant weight was obtained (Harbourne *et al.*, 1998). The percentage flavonoid was calculated thus:

$$\% \text{ flavonoids} = \frac{(\text{weight of beaker} + \text{residue}) - (\text{weight of beaker}) \times 100}{\text{weight of sample analysed}}$$

3.10.4 Terpenoids

Exactly 5 ml of extract was added into 2 ml of chloroform in a test tube, 1 ml of concentrated tetraoxosulphate (vi) acid (H_2SO_4) was also carefully added until a layer was formed**.** A reddish brown colouration at the interface showed positive results for the presence of terpenoids.

2 ml of chloroform was added to 5 ml of the sample and was transferred from assay tube to colorimetric cuvette containing methanol 95% (v/v); this was used as blank to read the absorbance at 538 nm. Exactly 200 ml of previously prepared linalool solution in methanol was added to 1.5 ml chloroform for the standard curve and serial dilution was done (dilution level 100 mg/200µl to 1 mg/200 µl linalool concentrations). For the serial dilution, total volume of 200 µl was made up by dilution of 95% (v/v) methanol.

$$\text{Terpenoid content, mg/100 g} = \frac{\text{Conc.of standard x absorbance of sample}}{\text{Arbsorbance of standard x weight of sample (g)}}$$

3.10.5 Cardiac Glycosides

Keller-Killani test

From the sample, 0.5 g was dissolved in 5 ml of water and 2 ml of glacial acetic acid solution containing one drop of ferric chloride solution was added to the mixture. Then 1 ml of conc. H_2SO_4 was added gently. A brown ring at the interface indicated presence of deoxysugar characteristic of cardenolides.

The quantity of cardiac glycosides was estimated as described by Osagie (1998). One mililitre (1ml) of 2% solution of 3, 5 dinitrosalicyclic acid (DNS) in methanol was added to 1ml of sample in a test tube and 1 ml of 5% aqueous sodium hydroxide was added. The mixture was boiled for 2 min. until brick-red precipitate was noted. Filter paper was weighed first, then boiled sample was filtered with the filter paper. The filter paper containing the absorbed residue was dried in an oven at 50^0C till dryness. The weight of the filter paper with residue was recorded. The cardiac glycoside was then calculated.

Calculation:

$$\% \text{ Cardiac glycoside} = \frac{(weight\ of\ filter\ paper + residue) - (weight\ of\ filter\ paper)\, x100}{weight\ of\ sample\ analyzed}$$

3.10.6 Alkaloids

Test for Alkaliods I

Two mililitres (2 ml) of hydrochloric acid was added to 5 ml of each sample; then 1 ml of Dregendoff's reagent was added to the mixture. Appearance of a red or orange precipitate indicated presence of alkaloid.

Test for Alkaliods II

Procedure:

Exactly 5 ml of 2% HCl was added to 1 ml of each extract in a test tube and heated in a water bath for 10 minutes. The solution was filtered and the filtrate was used for Wagner's reagent test as follows:

1ml of Wagner′s reagent was added to 10 ml of the filtrate in a test tube and mixed properly. This was observed for a colour change. A reddish brown precipitate indicated presence of alkaloids (Ejikeme *et al*., 2014).

Percentage of alkaloid present in the samples was determined using the method of Harbourne *et al*. (1998). 5 g of the extract from each sample was weighed into a 250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added. This was covered and allowed to stand for 4 h at 25^oC. This solution was filtered and the filtrate was concentrated to one- quarter of the original volume using a water bath. Concentrated ammonium hydroxide (NH_4OH) was added to the extract, dropwise, until precipitate was formed. The precipitate was collected and washed with 1% NH_4OH. The solution was filtered with pre-weighed filter paper. The residue on the filter paper was the alkaloid; it was dried in the oven at 80^oC. The percentage alkaloid content was then calculated as outlined by Harbourne *et al*. (1998).

Calculation:

$$\% \text{ weight of alkaloid} = \frac{(weight\ of\ filter\ paper + residue) - (weight\ of\ filter\ paper) x100}{weight\ of\ sample\ analyzed}$$

3.10.7 Phenols

Ferric Chloride Test

Into a test tube containing 1 ml of extract, 1 ml of 10% $FeCl_3$ was also added and mixed properly; it was observed for colour change. Greenish brown or black coloration indicated presence of phenolic nucleus.

Quantity of phenol in the sample was determined using spectrophotometric method. Two grams of sample was boiled with 50 ml of ethanol for 50 min. Using a pipette, 5 ml of the boiled sample was added into 50 ml volumetric flask and 10 ml of distilled water added. 2 ml of ammonium hydroxide (NH_40H) solution and 5 ml of concentrated pentanol were added to the mixture. The sample was made up to mark and left for 30 min. to react for the colour to develop. Absorbance of the solution was read using a spectrophotometer at 505 nm.

Total phenolic content was expressed as mg/100 g.

Total phenolic content was computed thus:

$$\% \text{ Phenol} = \frac{100}{W} X \frac{Au}{As} X \frac{C}{1000} X \frac{Vf}{Va} X D$$

Where: W = Weight of sample analyzed

Au = Absorbance of the test sample

As = Absorbance of the standard solution

C = Concentration of standard solution in mg/dl

Vf = Total filtrate volume

Va = Volume of extract analyzed

D = Dilution factor

3.10.8 Steroids

Liebermann-Burchard test was used to determine presence of steroids.

Two mililitres of acetic anhydride was added to 5 mg of sample and mixed with 2 ml of H_2SO_4. Change in colour, from violet to blue or green, indicated presence of steroids.

The steroid content was determined spectrophotometrically as described by Owiredu *et al*. (2013). Chloroform was added to 1 ml of the sample to make the volume up to 5 ml. Liberman-Burchard's reagent (0.5ml of conc. H_2SO_4 acid in 10 ml of acidic anhydride) was added and mixed. The mixture was covered with black paper and kept in the dark for 15 min. Green colour solution formed was measured using a spectrophotometer at 640 nm. Cholesterol was used as standard to construct a calibration curve.

$$\text{Steroid content, mg/100 g cholesterol} = \frac{\text{Conc. of standard x absorbance of sample}}{\text{Absorbance of standard x weight of sample (g)}}$$

3.10.9 Estimation of Phytate

The method of Unuofin, Otunola & Afulayan, (2017) was used to determine presence of phytate in the samples. Two grams of each sample was weighed into different 250 ml conical flasks. Each sample was then soaked in 100 ml of 2% concentrated hydrochloric acid (HCl) for 3hours. They were then filtered and 50 ml of each filtrate placed in 250ml beaker. 100 ml of distilled water was added. 10 ml of 0.3% ammonium thiocyanate was added as indicator and titrated with standard Iron III chloride solution containing 0.00195 g Iron per ml. Percentage of phytic acid was calculated using the formula:

$$\text{Phytic acid \%} = \frac{Titre\ value\ x\ 0.00195\ x\ 1.19\ x100}{2}$$

3.10.10 Determination of oxalate

This was determined based on the method described by Osagie (1998). This method involves digestion, oxalate precipitation and permanganate titration.

Digestion: 2 g of the sample was suspended in 190 ml of distilled water, using a 250 ml volumetric flask. Then 10 ml of 6M HCl was added and the suspension was digested for 1 h at 100ºC. This was then cooled and made up to 250 ml mark.

Oxalate precipitation: Two portions (125 ml) of the filtrate from digestion were measured into beakers and four (4) drops of methyl red indicator was added. Ammonium hydroxide (NH_4OH) solution was added in a dropwise manner until the test solution changed from salmon pink to a faint yellow colour. Each of the portions was then heated at 90ºC and filtered to remove precipitate containing ferrous ions. The filtrate was heated again to 90ºC and 10 ml of 5% Calcium chloride ($CaCl_2$) solution was added and gently stirred continuously. This was cooled and left to stand overnight at 25ºC. The solution was then centrifuged at 2500 rpm for 5 min. The supernatant was

decanted and the precipitate was completely dissolved in 10ml of 20% v/v tetraoxosulphate (VI) acid (H_2SO_4) solution.

Permanganate titration: Two grams (2 g) of sample, which was digested and filtered, was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and this was titrated against 0.05 M standardized Potassium permanganate ($KMnO_4$) solution to a faint pink colour which persisted for 30 s. The calcium oxalate content was calculated using the formula:

$$\text{Oxalate content (mg/100 g)} = \frac{T\ x\ (Vme)\ (Df)\ x\ 10}{ME\ x\ Mf}$$

Where T = titre value of $KMnO_4$,

Vme = volume – mass equivalent (1 ml of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid)

Df = dilution factor Vt/A, where Vt is the total volume of the titrate (300 ml) and A is the aliquot used (125 ml)

Me = molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction)

Mf = mass of sample used

3.11 Determination of *In Vitro* Antioxidant Activities

3.11.1 Determination of Radical Scavenging Activity against 2,2-Diphenyl-1-Picryihydrazyl (DPPH).

The method described by Lim and Quah (2007) was used to determine the free radical scavenging activity of the plant extracts against 2, 2- diphenyl -1-picrylhydrazyl radical. Various concentrations of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) were mixed vigorously in 5 ml of DPPH solution (0.1 mM in methanol) in test tubes. These were allowed to stand for 30 min. in a dark chamber at room temperature. A standard solution of antioxidant (ascorbic acid) was treated

in the same manner and incubated as well together with a reagent blank. At the end of the incubation process, the absorbance of the standard and extract was read with a spectrophotometer. Ability to decolourize DPPH was measured against blank at a wavelength of 517 nm. Percentage inhibition was then calculated thus:

$$\% \text{ inhibition} = \frac{(Abs\ Blank - Abs\ sample) \times 100}{Abs\ Blank}$$

Where Abs blank = absorbance of blank

Abs sample = absorbance of sample

3.11.2 Determination of Nitric Oxide Scavenging Acitivity

The method described by Panda, Raj, Shrivastava, and Prathani, (2009) and Alisi and Onyeze (2008) was used to measure the nitric oxide scavenging activity of the plant extracts. 4 ml of the extract was added at different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) to 1ml of sodium nitroprusside (SNP) solution (5mM) in test tubes, and the mixtures were incubated at 27^{0}C for 2 h. An aliquot (2 ml) of the incubated solution was taken and diluted with 1.2 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine-dihydrochloride). Absorbance of the chromophore, which was formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine-dihydrochloride, was immediately read at 550 nm and compared with the absorbance of standard ascorbic acid.

$$\text{Nitric oxide scavenging activity (\%)} = \frac{abs(control) - Abs(sample)}{Abs\ (control)} x100$$

Where Abs (control) = Absorbance of the control and

Abs (sample) = absorbance of the extract/standard.

3.11.3 Determination of hydrogen peroxide scavenging activity

This determination adopted the method of Ngonda (2013). A solution of hydrogen peroxide (2 mMol/l) was prepared in phosphate buffer (pH 7.4). Different concentrations of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) were each added to hydrogen peroxide solution (0.6 ml) in test tubes. Absorbance of hydrogen peroxide at 230 nm was read after 10 min. against a blank solution (only phosphate buffer) and compared with the standard (ascorbic acid). Hydrogen peroxide activity of the extracts was then calculated using the equation:

$$H_2O_2 \text{ activity } (\%) = \frac{Abs(control) - Abs(sample)}{Abs\ (control)} x100$$

Where, Abs (control) = absorbance of the control and

Abs (sample) =Absorbance of the extracts/standard

3.11.4 Determination of reducing power

Reducing power of the plants extracts was determined using the ferric reducing antioxidant power (FRAP) assay as recorded by Sofidiya, Odukoya, Familoni, & Inya-Agha, (2006). Various concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml) of the extracts were each added to 1 ml of deionized water, and were mixed with 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was left to incubate at 50^0C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added and it was centrifuged at 3000 rpm for 10 min. Thereafter, 2.5 ml of the decant was mixed with 0.5 ml of 0.1% $FeCl_3$. Absorbance was measured at 700 nm. Reducing power of the extracts was determined from the graph of optical density against concentration of the extracts. Reducing power (RP 0.5_{AU}) was determined as the concentration that gave 0.5 absorbance reading.

3.11.5 Total antioxidant activity

The total antioxidant capacities of the extracts were evaluated according to the phosphomolybdenum method described by Prieto, Pineda & Anguilar, (1999). Aliquots of 0.3 ml of sample solutions (100 mg/ml in ethanol) were each combined in 4 ml vials with 3ml of reagent solutions (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. Samples were cooled down at room temperature and the absorbance were measured at 695 nm. Ascorbic acid was used as standard and the antioxidant activity was expressed as mg of Ascorbic acid equivalent / g extract.

3.12 Acute Toxicity Evaluation

Acute toxicity was evaluated by determining the Median Lethal dose of the extracts. The median lethal dose (dose which can kill 50% of the experimental animals) (LD_{50}) of the crude aqueous and methanolic extracts of the roots, leaves and stems of *Chasmanthera dependens* and *Dictyandra arborescens* plants was determined using modified method of Lorke (1983) based on the description of Khan, Amupitan, Oyewale & Ndukwe (2015). This method involved two phases. 216 male Swiss albino mice were used for the two phases. In the first phase of each of the twelve (12) extracts, nine (9) male Swiss albino mice were divided into 3 groups of 3 mice each (n=3) and increasing doses of 10, 100 and 1000 mg/kg body weight of the extract were administered orally to groups 1, 2 and 3 respectively. These were observed for the first 4 hours and subsequently for 7 days for any signs of toxicity and mortality. These signs include pains, loss of appetite, difficulty in respiration, paw licking, weakness and death.

In the second phase (which was carried out when no death was recorded in the first phase), another one hundred and eight mice were used for the experiment as in phase one. For each of the twelve

(12) extracts, high doses of 1600, 2900 and 5000 mg/kg body weight of the extract were administered to another 3 groups of 3 fresh male Swiss albino mice (for each extract) through the same route. They were observed for 4 h for signs of toxicity and mortality, and subsequently for 7 days as in the first phase. This test was used to determine the safe dose of the extract that was administered to the experimental animals during the *in vivo* antimalarial test. Acute toxicity was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \, x \, D_{100}}$$

Where,

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

3.13 Sub-Acute Toxicity Test

This test was carried out as described by Ukoha, Okereke, Arunsi, Nwogu, Jack, Chukwudoruo, Nonyelum & Bello, (2017). Eithy-four (84) male albino rats weights ranging from 85 to 100 g were used for the study. They were divided into 14 groups of six (6) mice each (n=6). Group 1 received only feed and water (normal control), Group 2 received 200 mg/kg body weight of artesunate while groups 3 to 14 received 200 mg/kg body weight of the various extracts.These groups were labeled 1 to 14 as outlined below:

Group 1 - feed and water (normal control group)

Group 2 – artesunate (standard control group)

Group 3 – methanolic extract of *Dictyandra arborescens* root

Group 4 – methanolic extract of *Dictyandra arborescens* leaves

Group 5 – methanolic extract of *Dictyandra arborescens* stem

Group 6 - aqueous extract of *Dictyandra arborescens* root

Group 7 - aqueous extract of *Dictyandra arborescens* leaves

Group 8 - aqueous extract of *Dictyandra arborescens* stem

Group 9 - methanolic extract of *Chasmanthera dependens* root

Group 10 - methanolic extract of *Chasmanthera dependens* leaves

Group 11 - methanolic extract of *Chasmanthera dependens* stem

Group 12 - aqueous extract of *Chasmanthera dependens* root

Group 13 - aqueous extract of *Chasmanthera dependens* leaves

Group 14 - aqueous extract of *Chasmanthera dependens* stem

The standard drug and extracts were dissolved in distilled water to obtain the required dose level. Volume of drug given to the animals was calculated based on their body weight. The standard drug and extracts were administered to the experimental animals with the aid of an orogastric tube for fourteen (14) days (Ukoha *et al*., 2017). During this period, food and water were also given daily. Body weights of all the rats in the various groups were recorded before and after the experiment. On the 15th day, the rats were sacrificed by cervical decapitation and blood was collected by cardiac and ocular puncture using syringe and needle, into plane and ethylene diamine tetra-acetic (EDTA) bottles for heamatological and biochemical analyses respectively. Position of the heart was determined by palpating for the heartbeat. Blood samples in EDTA bottles were used for heamatological analyses while the samples in anticoagulant bottles were used for biochemical analyses. Blood samples for biochemical analyses were kept at room temperature for one hour to allow for maximum clotting. To collect the serum, blood was centrifuged at 3000 revolution per minute (rpm) for 10 min. Serum obtained was stored at -20°C in Eppendorf tubes for biochemical analysis.

3.14 Biochemical Analyses

Blood samples in the bottles without anticoagulants were used to determine biochemical parameters in this study. The biochemical parameters include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), proteins, and albumin.

3.14.1 Determination of Alanine Aminotransferase (ALT) Activity

Alanine aminotransferase in the serum was determined using Randox limited commercial kits as described by Yakubu, Richard-Haris, Shaibu, Abah & John, (2017).

Principle: Alanine aminotransferase (ALT) assay is based on the principle that pyruvate is formed from the reaction:

$$\alpha\text{- Oxoglutarate} + \text{L-alanine} \xrightarrow{ALT} \text{L-glutamate} + \text{pyruvate}$$

Concentration of pyruvate hydrazone formed with 2, 4-dinitrophenlyhydrazine was used to measure Alanine aminotransferase (ALT). The colour intensity was measured against the blank at 540 nm.

Procedure: Blank and sample test tubes were set up in duplicates. 0.1 ml of serum was pipetted into sample tubes. Buffer solution (0.5) ml containing phosphate buffer, L-alanine and α-Oxoglutarate was added. These mixtures were properly mixed and incubated at 37°C and pH of 7.4 for 30 min. To both tubes, reagent containing 0.5 ml of 2, 4-dinitrophenlyhydrazine was added, while 0.1 ml of sample was added to the sample blank tube. These tubes were thoroughly mixed and incubated for exactly 20 minutes at 25^{0}C. Five millilitres (5.0 ml) of Sodium hydroxide (NaOH) solution was added to each tube and mixed. Absorbance of the solution was read against the blank at 540 nm after 5 min.

3.14.2 Determination of Aspartate Aminotransferase (AST)

This was carried out as described by Yakubu *et al*. (2017).

Theory: Concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine is used to measure Aspartate Aminotransferase (AST). Colour intensity is measured against the blank at 546 nm.

Procedure: Blank and sample test tubes were set up in duplicates: 0.1 ml of serum was added into the sample test tubes using a pipette and 0.5 ml of reagent (1) containing phosphate buffer was added into both sample and blank tubes. The solutions were mixed properly and incubated at 37 ^{0}C and pH 7.4 for 30 min. Reagent (2) containing 2, 4-dinitrophenylhydrazine (0.5 ml) was added into all the test tubes while 0.1 ml of the sample was added into the blank tubes. The tubes were properly mixed and incubated for 20 min at 25°C; sodium hydroxide solution (5.0 ml) was then added to each tube and mixed thoroughly. Absorbance was read against the blank after 5 min. at 546 nm.

3.14.3 Determination of Alkaline phosphatase (ALP)

Principle: Alkaline phosphate reacts with a colourless substrate of phenolphthalein monophosphate to give phosphoric acid and phenolphthalein which turns pink at alkaline pH values (Yakubu *et al*., 2017).

P-nitrophenylphosphate + H_20 **--------→** ALP **------→** PO_4^{2-} + P-nitrophenol (pink at pH=9.6)

Procedure: The sample test tubes and blank were set up in duplicates and 0.05 ml of sample was added to the sample test tubes. 0.05 ml distilled water was pipetted into to the blank tube. Three mililitres (3.0 ml) of substrate was added into each tube respectively. This was then mixed thoroughly. Initial absorbance was taken at 405 nm. Absorbance of the sample and the blank were read again three (3) times at one minute intervals using a stop watch.

Calculation: alkaline phosphatase activity was calculated thus:

$$\text{Activity of ALP (in U/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3300$$

3.14.4 Estimation of Total Serum Proteins

Total serum proteins was estimated using the method of Oyinloye, Adenowo & Kappo, (2016).

Procedure: Three (3) test tubes, blank, standard and sample were labeled. To the sample tubes, 0.02 ml of serum was added; to the standard test tube, 0.02 ml of protein standard was added, and 0.02 ml of distilled water was added to the blank test tube. The principle of this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet complex. One millilitre (1.0 ml) of the protein reagent was added to the test tubes, mixed thoroughly and left to stand for 25 min. at room temperature (20-25° C). Absorbance was then taken at 540 nm.

$$\text{Total serum Proteins (in g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5 = \text{gram (g) of protein/dl}$$

3.14.5 Determination of Serum Albumin

Serum albumin was determined as outlined by Oyinloye *et al*. (2016) using Bromocresol Green. Albumin is measured by a dye-binding technique which is based on ability of albumin to form a stable complex with bromocresol green dye.

Principle: Serum albumin is measured by its quantitative binding to the indicator 3, 3, 5, 5, tetrabromo-m-cresolsulphonephthelein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm. The absorbance is directly proportional to the concentration of albumin in a given sample.

Procedure:

Wavelength	578 nm
Spectrophotometer	630 nm (600-650 nm)
Cuvette	1 cm light path
Incubation temperature	20-25°C
Measurement	Against reagent blank

In test tubes:

	Reagent blank	Standard	Sample
Distilled H_2O	0.01 ml	-	-
Standard (CAL)	-	0.01 ml	-
Serum or plasma	-	-	0.01 ml
BCG reagent (RI)	3.00 ml	3.00 ml	3.00 ml

The albumin concentration in the sample was calculated thus:

$$\text{Albumin Conc. (g/l or g/dl)} = \frac{\text{Abs sample} \times \text{Conc. of standard}}{\text{Abs standard}}$$

3.15 Haematological Analyses

Blood samples from experimental animals in the various groups for haematological analyses were collected in EDTA bottles. Method described by Ochei and Kolhatkar (2008) was used to determine the following haematological parameters: Red blood cells (RBC), White blood cells differential count (WBC), Haemoglobin concentration (HB), packed cell volume (PVC), mean corpuscular heamoglobin (MCH), mean corpuscular heamoglobin concentration (MCHC), neutrophils.

3.15.1 Determination of Erythrocyte (RBC) Count by Haemocytometry

This was determined using method of Ochei and Kolhatkar (2008). Blood specimen was diluted 1:200 using RBC diluting fluid; and number of cells was counted under high power (X40) objective lens in a counting chamber. Number of cells was calculated and reported as the number of red blood cells of whole blood.

Procedure: An aliquot (0.02 ml) of blood was added to 3.98 ml of sodium citrate and mixed properly. After 5 minutes, the first few drops were discarded by holding the pipette in a vertical position and the counting chamber was charged with the fluid. It was left to settle for 3 minutes. By Switching to low power (10x) objective, the centre large square with 25 small squares were adjusted to light and then adjusted to high power (40x) objective. Red blood cells in the four corner squares and one central square were counted.

Calculation

Total RBC / cu mm = number of cells counted x dilution factor

Area counted x depth of fluid where:

(1) Dilution = 1:200

(2) Area counted = 1/5 sq.mm

3.15.2 Determination of Total Leucocyte (WBC) Count by Haemocytometry

Total leucocyte count was determined by haemocytometry as described by Ochei and Kolhatkar (2008). Glacial acetic acid causes lysis of the red cells while the gentian violet slightly stains the nuclei of the leucocytes. Blood specimen was diluted 1:200 using a WBC pipette. The diluting fluid and the cells were counted under low power microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cells/cu.mm of whole blood.

Procedure: An aliquot (0.02 ml) of blood was added to 0.38 ml of diluting fluid (Acetic acid, tinged with gentian violet) and mixed. The counting chamber was charged with the well-mixed diluted blood (after discarding the first five drops) using a pipette. Cells were left to settle in a moist chamber for 3 minutes. The four corners of the chamber were viewed under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Calculation

Total WBC / cu mm = Number of cells counted x dilution factor

Area counted x depth of fluid

Where:

(1) Dilution = 1:200

(2) Area counted = 1/5 sq.mm

(3) Depth of fluid= 1/10 mm

(4) Number of red cells counted = N x 200

3.15.3 Estimation of Packed Cell Volume (PCV)

Method described by Ochei and Kolhatkar (2008) was used to determine PCV. Blood sample was taken with a heparinised capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhaematocrit centrifuge and spun at 12,000 rpm for 5 minutes. Spunned tubes were placed in a specially designed scale and PCV was read as a percentage thus:

$$\text{PCV \%} = \frac{\text{Packed RBC column height}}{\text{Total blood volume height}} \times 100$$

3.15.4 Determination of Haemoglobin (Hb) Concentration

This was carried out using cyanomethaglobin method as described by Ochei and Kolhatkar (2008).

Principle: Haemoglobin when mixed with Drabkin's solution, containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate forms methaemoglobin which is then converted to cyanmethaemoglobin by the cyanide. The cyanmethaemoglobin produces a colour which is measured.

Procedure: 0.02 ml of whole blood was added to 5 ml of Drabkin's solution in a test tube (1:250 dilution). This was mixed properly, left to stand for 10 min. and the absorbance was read colorimetrically at 540 nm using Drabkin's solution as a blank.

Calculation: Grams of haemoglobin per 100 ml of blood was determined as haemoglobin

3.15.5 Determination of mean corpuscular haemoglobin (MCH)

This is the weight of haemoglobin in the average erythrocyte count. It was calculated as

MCH (pg) = Hb/RBC x 10

3.15.6 Determination of mean corpuscular haemoglobin concentration (MCHC)

This is the average weight of haemoglobin in a measured dilution (1:250). It was calculated as

MCHC (g/dl) =Hb/Hct x 100

3.15.7 Determination of neutrophils and lymphocytes

This was determined by differential leucocyte count (DLC) as described by Ochei and Kolhatkar (2008). A drop of the whole blood was placed on one end of a glass slide and another slide was used to spread the blood film. The blood film was allowed to dry and labeled at the thick end. The slide was then fixed in absolute methanol for 5 min. It was then removed from the alcohol and allowed to dry; stained with giemsa and allowed to stand for 40 min. The slides were then removed

from the stain, washed with buffered distilled water and allowed to dry. A drop of cider oil was placed over the thin end of the smear and examined under oil immersion lens. Examination started at the thin end of the smear. The DLC was carried out using the 4 field method. At least, 100 leucocytes were counted in the blood film and relative number of each type of WBC in relation to the total WBC was calculated. The absolute number of each type of WBC was obtained by multiplying the portion of that type of cell by the total WBC count.

Neutrophil was calculated as: $Ne\ (\%) = \frac{\text{Number of neutrophils}}{\text{Total number of WBC}} \times 100$

Lymphocytes were calculated as $Ly\ (\%) = \frac{\text{Number of lymphocytes}}{\text{Total number of WBC}} \times 100$

3.16 *In Vivo* Antimalarial Test of the Crude Extracts

This test was carried out using the 4-day curative test described by David, Philip, Simon & Solomon, (2004) and Peter & Anatoli, (1998) using rodent malaria parasite, *Plasmodium berghei*. Ninety (90) male Swiss albino mice were used for this study. They were shared into 15 groups of six (6) mice each (n=6) according to their body weight as described by Khan *et al*. (2015). These groups were labeled 1 to 15 as follows:

Group 1 - feed and water (normal control group)

Group 2 – infected and treated with artesunate (standard control group)

Group 3 – infected but no treatment (negative control group)

Group 4 – infected and treated with methanolic extract of *Dictyandra arborescens* root

Group 5 – infected and treated with methanolic extract of *Dictyandra arborescens* leaves

Group 6 - infected and treated with methanolic extract of *Dictyandra arborescens* stem

Group 7 - infected and treated with aqueous extract of *Dictyandra arborescens* root

Group 8 - infected and treated with aqueous extract of *Dictyandra arborescens* leaves

Group 9 - infected and treated with aqueous extract of *Dictyandra arborescens* stem

Group 10 - infected and treated with methanolic extract of *Chasmanthera dependens* root

Group 11 - infected and treated with methanolic extract of *Chasmanthera dependens* leaves

Group 12 - infected and treated with methanolic extract of *Chasmanthera dependens* stem

Group 13 - infected and treated with aqueous extract of *Chasmanthera dependens* root

Group 14 - infected and treated with aqueous extract of *Chasmanthera dependens* leaves

Group 15 - infected and treated with aqueous extract of *Chasmanthera dependens* stem

Experimental animals in groups 2 to 15 were inoculated with *Plasmodium berghei* on day zero (D_0) through intraperitoneal route. Before the experimental animals were inoculated with infected blood from the donor mice, the parasitized blood from the donor mice was first diluted with normal saline (0.9% sodium chloride) to reduce parasite count as described by Kabiru, Okolie, Muhammad & Ogbadoyi, 2012. The dilution was made by adding seven (7) drops of the parasitized blood in 10ml of normal saline. This inoculum was prepared such that each 0.2ml contained approximately 1 x 10^7 parasitized erythrocytes. The animals were then injected with 0.2ml of this suspension. These test animals were marked with picric acid (2,4,6 trinitrophenol) dye on various parts of their bodies for easy identification during administration of drug and extracts. The test animals were left for seventy-two (72) h after infection with the malaria parasites. This was to ensure that parasitemia was fully established in the mice. Level of parasitemia was determined after three days (72 hours). Treatment commenced on day 3 (D_3), (72 h after infection), by oral route and this lasted for four (4) consecutive days (Ene *et al*., 2013; David *et al*., 2004; Peter & Anatoli, 1998). During treatment, animals were carefully handled as described by Ahatty (2012). All animals in the treatment groups received 200mg/kg body weight

of the standard antimalarial drug (artesunate) and 200 mg/kg body weight of the various extracts once daily for four consecutive days. The dose level of 200 mg/kg body weight was chosen based on the result of the acute toxicity test which was up to 5000 mg/kg body weight for most of the extracts and 3808mg/kg body weight for the aqueous and methanol extracts of *Chasmanthera dependens* stem. The extracts were dissolved in distilled water to obtain the required dose level. Volume of drug given was calculated based on the body weight of the animals. The level of parasitemia was monitored in all the groups on day 7 (4th day of treatment) and on day 14 (7 days post treatment) as described by Arrey, Okalebo, Ayong, Agbor & Guantai, (2014) and the results were compared with that of the untreated group. Thick and thin smears of blood films were made from a cut on the tip of the tail of the mice. The smears were fixed with methanol for 15 min and stained with 10% giemsa (a reagent that stains the malaria parasite) for 25 min. The film was thereafter washed off with phosphate buffer (pH 7.2) and left to dry. This was examined under the light microscope (magnification =X100) under a drop of immersion oil to access the level of parasitemia. Percentage parasitemia in pre-treated animals was calculated on day 3 using the method described by Adeyemo-Salami, Farombi & Ademowo, (2014) thus:

$$\text{Percentage parasitemia in pre-treated animals} = \frac{\text{No of Parasitized cells}}{\text{total number of red blood cells}} \, X \, \frac{100}{1}$$

While the percentage parasitemia in treated animals was calculated based on the method outlined by Iwalewa *et al*. (1997), thus:

$$\text{\% Parasitemia} = \frac{\text{No of Parasitemia in Treated}}{\text{No of Parasitemia in control}} \, X \, \frac{100}{1}$$

Determination of percentage body weight of the experimental animals after treatment

The body weight of the experimental animals were calculated on the 14th day to determine the effects of the plants' extracts, thus:

$$\text{Percentage body weight} = \frac{final\ body\ weight - initial\ body\ weight}{initial\ body\ weight}\ X\ \frac{100}{1}$$

Determination of percentage survival of the experimental animals after treatment

Survival rate of the experimental animals was monitored until the 14th day. This was then expressed in percentage thus:

$$\text{Percentage survival} = \frac{Number\ of\ animals\ that\ survived}{total\ number\ of\ animals\ in\ the\ group}\ X\ \frac{100}{1}$$

3.17 Isolation of Bioactive Compound(s)

Isolation of bioactive compounds was conducted according to bioassay-guided isolation protocol (Cseke, Kirakosyan & Kaufman, 2006). The most active extract was subjected to separation using column chromatography to obtain different fractions of the mixture.

3.17.1 Column Chromatographic separation of methanol extract of *D.arborescens* root

The most active crude extract (methanol extract of *D. arborescens* root) was subjected to silica gel (70-230 mesh) column chromatographic separation using slurry method (Duru & Duru, 2017). Three hundred grams (300 g) of silica gel was mixed with hexane to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then gently poured into a glass column. The sample to load on the column was prepared by dissolving 50 g of the extract in 100 ml of methanol. 25 g of silica gel was added to the solution and mixed thoroughly using a glass stirring rod. The mixture was allowed to dry at room

temperature to a free flowing powder. The dried silica- extract mixture was introduced into the loaded column. The column was first eluted with hexane as the mobile phase with the polarity increasing by 10% increments of chloroform. After getting to 100 % chloroform, the polarity was further adjusted by increasing with 10 % increments of methanol. The eluting solvent was collected at 150 ml volumes and each allowed to evaporate to 5 ml volume at room temperature (25 ^{o}C) to concentrate any eluted compound.

3.18 Bioassay-Guided Antimalarial Evaluation of Eluates from Column Chromatography

The resulting eluates were screened for antimalarial activity using the 4-day curative test by repeating the procedure in **3.16**. Twenty four (24) male Swiss albino mice weighing 20 g were used for this study. They were shared into 8 groups of three (3) mice each (n=3). These groups were labeled 1 to 8 thus:

Group 1 - feed and water (normal control group)

Group 2 – infected and treated with artesunate (standard control group)

Group 3 – infected but no treatment (negative control group)

Group 4 – infected and treated with Eluate A [100% hexane (1)]

Group 5 – infected and treated with Eluate B [100% hexane (2)]

Group 6 - infected and treated with Eluate C [50% hexane **:** 50% chloroform (1:1)]

Group 7 - infected and treated with Eluate D [40% hexane : 60% chloroform (2:3)]

Group 8 - infected and treated with Eluate E [80% chloroform : 20% methanol (4:1)]

All test animals in the treatment groups received 100 mg/kg body weight of the standard antimalarial drug (artesunate) and 100 mg/kg body weight of the various eluates once daily for four (4) consecutive days. Level of parasitemia was monitored in all the groups on the 7th day (4

days after treatment) and on the 14th day (7 days post treatment). Results were compared with those of the untreated group as described in 3.16.

Eluate A [100% hexane (1)] which showed the highest antimalarial activity was sub-fractionated using column chromatography to get two purer eluates coded **A** and **B**.

3.19 Characterization of the Eluates with the Highest Antimalarial Activity

Phytochemicals in the two eluates obtained from hexane fraction, H_{100} (1), were determined using Gas chromatography, flame ionization detector (GC-FID) and partially characterized using spectrometric techniques, such as Fourier transform Infrared (FTIR) spectroscopy and Gas chromatography- mass spectrometry (GC-MS) as described by Nagarajan and Kumar (2017) and Pkkirisamy, Kalakandan and Ravichandran (2017). GC-FID was used to determine the phytochemicals present in the eluates. Fourier Transform infrared (FTIR) spectroscopy was used to characterize the functional groups present in the eluates while Mass spectrometry (MS) was used to determine the compounds present in the eluates.

3.19.1 GC-FID analysis of the eluates

Phytochemicals in the crude hexane fractions (A and B) were identified using BUCK M910 Gas Chromatograph equipped with a flame ionization detector (GC-FID) as described by Duru, (2019). Exactly 1 g of the pulverized sample was placed in a test tube, 15 mL of ethanol and 10 mL of 50 % w/v potassium hydroxide (KOH) were added. The solution was placed in a water bath for 60 min at 60 ^{o}C. Content of the test tube was then carefully transferred into a separating funnel. 10 mL of cold water, 10 mL of hot water, 20 mL of ethanol and 3 mL of hexane were used to rinse the test tube into the funnel. The extract in the funnel was rinsed three times using 10ml of 10 %

v/v ethanol. This was dried out using anhydrous sodium sulphate and the solvent was evaporated. A little quantity of the extract was made soluble in 100 µl pyridine and 20 µl was placed in a vial on the GC equipment for phytochemical analysis.

The identification of phytochemicals in the crude extract was carried out with a BUCK M910 Gas Chromatograph fitted with a flame ionization detector (GC-FID). A RESTEK 15 m MXT-1 column (15 m × 250 µm × 0.15 µm) was used. The injector temperature was 280 ^{o}C with splitless injection of 2 µl of extract and a linear velocity of 30 cms^{-1}. The carrier gas was Helium 5.0 Pas with a flow rate of 40 $mLmin^{-1}$. Initial operating temperature was 80 ^{o}C, and increased to 330 ^{o}C at a rate of 5 ^{o}C min^{-1} and kept at this temperature for 5 min. Operating temperature of the detector was 320 ^{o}C. The identification of compounds was based on the retention time which is determined using authentic standards. The integrated peak area is directly converted to concentrations in a user defined unit, such as $\mu g/m^3$. The calculations are performed by the data acquisition and processing station. All processed data were automatically compiled and reported through a data report function incorporated in the data station. The reported data includes the compound, name, retention time, and concentration.

3.19.2 Fourier Transform infrared (FTIR) spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) is a powerful tool for identifying the types of functional groups present in unknown compounds. The wavelength of light absorbed is characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined.

FTIR was carried out as described by Nagarajan & Kumar (2017). Dried powder of samples of the unknown compounds was used for FTIR analysis. 10mg of dried sample was encapsulated in 100

mg of KBr pellet in order to prepare transluscent sample discs. Each sample was loaded in FTIR spectroscope (Agilent Cary 630 Fourier transform infrared spectrophotometer), with a scan range of 400 to 4000 cm^{-1} to obtain the spectra wavelength.

3.19.3 Gas Chromatography-Mass spectrometry (GC-MS) analysis

The sample was analyzed as described by Pkkirisamy *et al*., 2017 using agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system as follows: Hp 5-MS capillary standard non-polar column, dimension: 30 M, ID: 0.25 mm, Film thickness: 0.25 μm. Flow rate of mobile phase (carrier gas – Helium) was set at 1.0 ml/min. in the gas chromatography part, temperature programme (oven temperature) was 40^0C raised to 250^0C at 5^0C/min and injection volume was 1μl. Samples dissolved in methanol were run fully scan at a range of 40 -650 m/z and the results were compared and interpreted using National Institute Standard and Technology (NIST) Mass Spectral library database search programme with over 62,000 patterns for identifying chemical compounds.

3.19.4 Molecular docking analysis

The chemical compounds obtained from GC-MS analysis of the two eluates (A and B) were subjected to molecular docking with two antimalarial protein targets as described by Tian, Chen, Lei, Zhao and Liang, (2018). These molecular target proteins- *Plasmodium berghei* lactate dehydrogense (*Pb*LDH) and Plasmepsin II with protein databank identities (PDB ID): **1OC4** and **1SME** were identified from literature and used as drug target in this study. Lactate dehydrogenase (1OC4) is a key enzyme that catalyses the synthesis of lactate from pyruvate in *Plasmodium* species while Plasmepsin II (1SME) is a hemoglobin-degrading enzyme in *Plasmodium* species. The 3D structures of the protein molecules were obtained from protein databank (www.rcsb.org).

The existing ligands and water molecules were removed and hydrogen molecules were added. The SDF (structure-data file) structures of the compounds and standard ligand i.e artesunate were identified and downloaded from the Pubchem database. They were minimized in Pyrx virtual screening tool, using Universal Force Field at 200 steps. Chemical compounds present in the two eluates (**A** and **B**) of hexane fraction of methanol extract of *D.arborescens* roots were then converted to AutoDock ligands and used for the docking analysis. Computed Atlas for Surface Topography of Proteins (CASTp) was used to view the active sites and amino acid residues around the lagest active site. Multiple docking of the ligands on specified *Plasmodium berghei* lactate dehydrogense and Plasmepsin II protein binding pockets was done with AutoDockVina in Pyrx software (version 0.8) (Trott *et al*. 2010). The binding free energies of the compounds on the protein targets were obtained after the docking process. Biovia Discovery studio 4.5 was then used to visualize the interactions between the protein-ligand complexes after the docking process.

3.19.5 Absorption, Distribution, Metabolism, Elimination and Toxicity (ADMET) Analysis

The most potent bioactive compounds were chosen and sent to the SwissADMET server to examine their drug-like properties and compare them with those of the control drug as described by Daina, Michelin and Zoete, (2017).

3.20 Calculation of Percentage Yield of Extracts

Yield of extract is the quantity of extract obtained after extraction. This was expressed in percentage to give the percentage yield using the formular:

Percentage yield =

$$\frac{\text{weight of crude extract (g)}}{weight\ of\ plant\ material\ (g)}\ X\ \frac{100}{1}$$

3.21 Statistical Analyses

Tables, Graphs and charts were used to present results obtained in this study. Data obtained were presented as mean ± standard error of mean (SEM) and were subjected to Analysis of Variance (ANOVA) procedure using statistical analysis system (SAS) package version 20 software. Mean differences were subjected to Duncan's multiple range test (DMRT) at significance level of $p < 0.05$.

The software Table curve 2D systat version 5.01, USA and Sigma plot 10. 0 Systat USA were used for mathematical modelling of data obtained for *in vitro* antioxidant activities. Percentage inhibition was calculated relative to control using equation 1. Inhibition data (mean values obtained from triplicate determinations) generated were fitted into appropriate non-linear kinetic equations (Alisi *et al.*, 2011) which include Sigmoid (a,b,c) (Eqn 2), logistic dose response model LDR (a,b,c) (eqn 3), LDR (a,b,c,d) (eqn 4), and Weibull cumulative (a,b,c,d) (eqn 5). In the mathematical modeling of data, asymmetric non-linear equations with highest Pearson correlation coefficient (R^2) values and tolerance were selected for fitting data.

The dose-response data were fitted to obtain their respective IC_{50} values which is the concentrations of the extract that inhibited 50% of respective radicals.

Equations:

$$\% \, Inhibition = \left[\frac{Control_{ABS} - Test_{ABS}}{Control_{ABS}}\right] \times 100 \qquad 1$$

Sigmoid a,b,c model

$$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)} \qquad 2$$

Where: x is the concentration of the extract, y is the % Inhibition, a is the maximum response (of control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

Logistic dose response model (a, b,c) and (a,b,c,d)

$$y = \frac{a}{1 + \left(\frac{x}{b}\right)^{c}} \qquad 3$$

Where: x is the concentration of the extract, y is the % Inhibition, a is the maximum response (of control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^{d}} \qquad 4$$

Where: x is the concentration of the extract, y is the % Inhibition, a is the maximum response (of control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}, d is a constant that determines the degree of asymmetry.

Weibull cumulative (a, b, c, d)

$$y = a\left[1 - exp\left[-\left[\frac{x + c(In\ 2)^{1/d} - b}{c}\right]^{d}\right]\right] \qquad 5$$

Where: x is the concentration of the extract, a is the maximum response (of control), b is the IC_{50}, c is parameter determining the relative slope at $IC_{50,}$ d is a constant that determines the degree of asymmetry.

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percentage yield of extracts

The Percentage yield of extracts is represented in Table 4.1. The highest yield of extract was obtained from the methanolic extract of *Dictyandra arborescens* leaves (24.9%); this was followed by methanol extract of *Chasmanthera dependens* leaves (22.6%), methanolic extract of *Chasmanthera dependens* root (22.4%), aqueous extract of *Dictyandra arborescens* leaves (21.3%), and methanol extracts of *Dictyandra arborescens* roots (20.6%) respectively. The least percentage yield (8.1%) was obtained from aqueous extract of *Dictyandra arborescens* stem followed by the aqueous and methanol extracts of *Chasmanthera dependens* stem whose yield were 9.2% and 10.1% respectively. These were lower than the 12.3% obtained from methanol extract of *Dictyandra arborescens* stem. Percentage yield of the aqueous extracts of *Chasmanthera dependens* roots, leaves and *Dictyandra arborescens* roots were 18.3%, 17.5% and 16.7% respectively. Results obtained after extraction showed that yield of extract obtained from methanol extracts were more than those of the aqueous extracts.

Table 4.1: Percentage yield of extracts from the roots, stems and leaves of *Chasmanthera dependens* and *Dictyandra arborescens*

Plant parts	Solvent (ml)	Percentage yield (% w/w)
D.arborescens leaves	Methanol	24.9
D.arborescens leaves	Aqueous	21.3
D.arborescens root	Methanol	20.6
D.arborescens root	Aqueous	16.7
D.arborescens stem	Methanol	12.3
D.arborescens stem	Aqueous	8.1
C.dependens leaves	Methanol	22.6
C.dependens leaves	Aqueous	17.5
C.dependens root	Methanol	22.4
C.dependens root	Aqueous	18.3
C.dependens stem	Methanol	10.1
C.dependens stem	Aqueous	9.2

4.1.2 Qualitative and Quantitative Phytochemical Results

Results of phytochemical analyses of the leaves, stems and roots of *Chasmanthera dependens* and *Dictyandra arborescens* plants showed that the two plants are rich in various phytochemicals, such as flavonoids, alkaloids, tannins, steroids, saponins, phenols, oxalate, cardiac glycosides, terpenoids, phytate etc (Table 4.2). For *Dictyandra aborescens*, values of some of the phytochemicals were higher (+++) in the methanol extracts of the leaves, stem and roots compared with the aqueous extracts. For instance, values of tannins were high (+++) in the methanol extracts of the leaves and roots but were moderate (++) in the aqueous extracts of the same parts of the plants. Terpenoids were high (+++) in methanol and aqueous extracts of the leaves and roots but were moderate (++) in the stem for both extracts. Phenols were high (+++) in methanol extracts of

the leaves and roots but were moderate (++) in aqueous extracts of both plant parts. Alkaloids, oxalate and phytate values were either low or moderately present in aqueous and methanol extracts of the leaves, stem and roots of *Dictyandra aborescens* plant. However, oxalate was not detected in both methanol and aqueous extracts of the root of *Dictyandra aborescens* while phenol was not detected in aqueous extract of the stem of this plant.

For *Chasmanthera dependens*, most of the phytochemicals were present in the different parts (leaves, stem and roots) of the plant in varying amounts (Table 4.3). Terpenoids were high (+++) in the methanol and aqueous extracts of the leaves and roots but were moderate (++) in the stem. Tannin was high (+++) in methanol extract of the leaves and roots of this plant. It was, however, moderate (++) in the aqueous extracts of the same plant parts. Other phytochemicals, saponins, alkaloids, phenols, flavonoids, oxalate and phytate were either present in low (+) or moderate (++) concentrations in the methanol and aqueous extracts of various parts of *Chasmanthera dependens* used in this study, except aqueous extract of the stem where alkaloids and phenols were not detected. Qualitative phytochemical analyses of these plants parts indicated that methanol and water are good solvents for the extraction of therapeutic compounds from the two medicinal plants under study; however, methanol extracted more than water (aqueous) solvent.

Quantitative analyses of the various phytochemicals present in the methanol and aqueous extracts of the leaves, stem and roots of the two plants is presented in Tables 4.4 and 4.5. The most abundant phytochemicals in the leaves, stem and roots of *D.arborescens* plant were tannins (31.24±0.03%), saponins (13.23±0.02%), and flavonoids (22.99±0.39%) respectively while those of *C.dependens* were tannins (37.66±0.02%) and terpenoids (80.0±0.12 mg/100g).

Table 4.2: Qualitative phytochemical contents of the aqueous and methanolic extracts of the leaves, stems and roots of *Dictyandra arborescens*

	Methanolic Extracts			Aqueous Extracts		
Phytochemical	**DAL**	**DAS**	**DAR**	**DAL**	**DAS**	**DAR**
Tannins	+++	++	+++	++	++	++
Saponins	+++	++	++	++	+	+
Alkaloids	+	+	++	++	+	+
Cardiac Glycosides	+++	++	++	++	++	++
Terpenoids	+++	++	+++	+++	++	+++
Steroids	+	+++	+	+	+++	+
Phenols	+++	+	+++	++	ND	++
Flavonoids	++	+	++	+	+	+
Oxalate	+	++	ND	+	+	ND
Phytate	+	++	+	+	+	+

Key: +++ = High, ++ = moderate, + = low, ND = Not Detected

DAL =*Dictyandra aborescens* leaves, DAS = *Dictyandra aborescens* stem, DAR = *Dictyandra aborescens* roots

Table 4.3: Qualitative phytochemical contents of the aqueous and methanolic extracts of the leaves, stems and roots of *Chasmanthera dependens*

	Methanolic Extracts			Aqueous Extracts		
Phytochemicals	**CDL**	**CDS**	**CDR**	**CDL**	**CDS**	**CDR**
Tannins	+++	++	+++	++	++	++
Saponins	+	++	+	+	++	+
Alkaloids	++	+	++	++	ND	++
Cardiac Glycosides	++	++	+++	++	++	++
Terpenoids	+++	++	+++	+++	++	+++
Steroids	+	++	+++	+	+++	+++
Phenols	+	+	+	+	ND	+
Flavonoids	++	+	++	+	+	++
Oxalate	++	+	++	++	+	++
Phytate	++	++	++	++	+	++

Key: +++ = High, ++ = moderate, + = low, ND = Not Detected

CDL = *Chasmanthera dependens* leaves, CDS = *Chasmanthera dependens* stem

CDR = *Chasmanthera dependens* root.

Table 4.4: Quantitative determination of phytochemicals in the methanolic and aqueous extracts of the leaves, stems and roots of *Dictyandra arborescens*

	Methanolic Extracts			Aqueous Extracts		
Phytochemicals	**DAL**	**DAS**	**DAR**	**DAL**	**DAS**	**DAR**
Tannins (%)	31.24±0.03	12.83±0.03	21.15±0.03	16.10±0.13	10.60±0.07	14.74±0.22
Saponins (%)	20.60±0.05	13.23±0.02	15.65±0.09	18.90±0.05	3.80±0.05	3.10±0.03
Alkaloids (%)	3.16±0.02	1.66±0.03	8.95±0.05	1.89±0.11	1.20±0.01	4.00±0.22
Cardiac Glycosides (%)	27.0±2.02	11.79±0.13	14.79±0.05	15.80±0.20	9.87±0.16	11.92±0.09
Terpenoids (mg/100g)	386.80±0.05	50.40±0.12	148.16±0.03	359.41±0.13	48.39±0.18	148.20±0.21
Steroids (mg/100g)	31.04±0.05	129.92±0.65	50.94±0.06	29.22±0.27	127.80 ±0.14	42.43±0.27
Phenols (mg/g)	11.26±0.08	0.66±0.11	11.16±0.07	7.16±0.02	_	8.02±0.05
Flavonoids (%)	28.71±0.29	3.22±0.10	22.99±0.39	7.25±0.31	3.08±0.06	5.61±0.13
Oxalate (mg/100g)	1.9 $\pm 0.06 \times 10^4$	5.2 $\pm 0.06 \times 10^4$	_	1.20 $\pm 0.09 \times 10^4$	3.8 $\pm 0.02 \times 10^4$	_
Phytate (%)	2.47±0.18	6.50±0.14	3.25±0.14	1.17±0.12	3.05±0.23	2.20±0.09

Values are mean ± standard error of mean (SEM) of triplicate determinations

Key: DAL =*Dictyandra aborescens* leaves, DAS = *Dictyandra aborescens* stem,

DAR = *Dictyandra aborescens* roots

Table 4.5: Quantitative determination of phytochemicals in the methanolic and aqueous extracts of the leaves, stems and roots of *Chasmanthera dependens*

	Methanolic Extracts			Aqueous Extracts		
Phytochemical	**CDL**	**CDS**	**CDR**	**CDL**	**CDS**	**CDR**
Tannins (%)	37.66±0.02	15.17±0.04	25.31±0.40	18.80±0.42	12.74±0.16	14.60±0.06
Saponins (%)	3.18±0.04	12.61±0.06	6.32±0.06	3.12±0.03	11.84±0.03	5.70±0.05
Alkaloids (%)	6.01±0.12	1.51±0.01	6.93±0.05	5.90±0.07	_	4.01±0.05
Cardiac Glycosides(%)	16.17±0.12	16.31±0.08	24.85±0.14	15.05±0.25	13.24±0.15	18.34±0.20
Terpenoids (mg/100g)	80.0±0.12	42.24±0.03	75.60±0.10	62.08±0.06	31.30±0.06	65.54±0.18
Steroids (mg/100g)	13.24±0.15	49.02±0.25	69.56±0.12	9.02±0.22	54.31±0.27	58.93±0.07
Phenols (mg/g)	0.17±0.01	0.21±0.04	0.38±0.07	0.19±0.02	_	0.30±0.05
Flavonoids(%)	5.65±0.20	3.43±0.08	8.30±0.10	3.00±0.07	3.21±0.19	9.41±0.12
Oxalate (mg/100g)	4.7±0.23	2.9±0.17	5.7±0.25	4.10±0.05	1.80±0.10	6.48±0.10
Phytate(%)	9.40±0.25	8.01±0.12	8.93±0.47	8.60±0.35	3.33±0.20	6.74±0.04

Values are mean ± standard error of mean (SEM) of triplicate determinations

Key: CDL = *Chasmanthera dependens* leaves, CDS = *Chasmanthera dependens* stem

CDR = *Chasmanthera dependens* root.

4.1.3 *In vitro* antioxidant activities

4.1.3.1 Effects of crude aqueous and methanol extracts of leaves, stem and roots of *D.arborescens*, *C.dependens* and Ascorbic acid (standard) on Scavenging of 2,2-Diphenyl-1-Picryhydrazyl (DPPH) Radical.

Figure 4.1 shows the DPPH radical scavenging properties of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid (AA) standard within the tested concentrations (0.5 mg/ml- 2.5 mg/ml). Activities were dose-dependent as increase in concentration of extracts increased DPPH scavenging activities. Aqueous extract of *D.arborescens* root (DARA) exhibited the highest DPPH scavenging activity, with inhibitory concentration (IC_{50}) value of 1.19 ± 0.08 mg/ml. Activities of the extracts were significantly ($p<0.05$) lower compared with the standard antioxidant. Results obtained showed that DPPH radical scavenging activities of the aqueous and methanol extracts were mostly sigmoidal; the extracts demonstrated effective inhibitory effect against DPPH radical. Mathematical modeling of results is presented on Table 4.6, showing threshold Inhibitory concentrations (IC_{50}) of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and Ascorbic acid standard on DPPH radicals. R^2 value ranged from 0.934 to 0.975, indicating a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal pattern, model (sigmoid a, b, c) with the equation shown in Table 4.6. IC_{50} values ranged from 1.66 ± 0.27, 1.61 ± 0.19, 1.26 ± 0.10, 1.19 ± 0.08, 1.45 ± 0.09, 1.34 ± 0.12 to 0.58 ± 0.05 mg/ml for the extracts DARM, DALM, DASM, DARA, DALA, DASA and Ascorbic Acid respectively. Furthermore, comparison of evaluated IC_{50} values shows that DPPH radical scavenging effectiveness was in the order AA > DARA > DASM > DASA > DALA > DALM > DARM.

Activities of the extracts were also dose-dependent for *C.dependens*. Aqueous extract of the root of this plant showed the highest activity, with an IC_{50} value of 1.10 ± 0.12 mg/ml (Table 4.7). DPPH radical scavenging activities of the extracts were significantly ($p<0.05$) lower than that of the standard antioxidant whose IC_{50} value was 0.58±0.05 mg/ml. Results obtained from this study shows that DPPH radical scavenging property of the aqueous and methanol extracts were also largely sigmoidal, extracts demonstrated effective inhibitory effect against DPPH radicals. Mathematical modeling of results is presented on Table 4.7; R^2 value ranged from 0.934 to 0.975, indicating a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal pattern, model (sigmoid a,b,c) with the equation shown in Table 4.7; inhibitory concentration values were 1.81 ± 0.32, 1.44 ± 0.14, 1.52 ± 0.10, 1.10 ± 0.12, 1.81 ± 0.32, 1.51 ± 0.14 and 0.58 ± 0.05 mg/ml for the extracts, CDRM, CDLM, CDSM, CDRA, CDLA, CDSA and AA respectively. Comparative evaluation of IC_{50} values shows that DPPH radical scavenging activities followed the order AA > CDRA >CDLM > CDSA > CDSM > CDLA > CDRM.

Table 4.6: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* and Ascorbic acid standard on DPPH radicals

Extracts	Threshold inhibitory concentration (*IC_{50})	Mathematical Model	Equation	R^2
DARM	1.66 ± 0.27	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.948
DALM	1.61 ± 0.19	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.959
DASM	1.26 ± 0.10	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.956
DARA	1.19 ± 0.08	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.964
DALA	1.45 ± 0.09	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.975
DASA	1.34 ± 0.12	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.959
AA	0.58 ± 0.05	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.934

* IC_{50} is the concentration of the extract or standard that scavenged 50% of the generated free radicals.

Key: DARM= *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, AA =Ascorbic acid

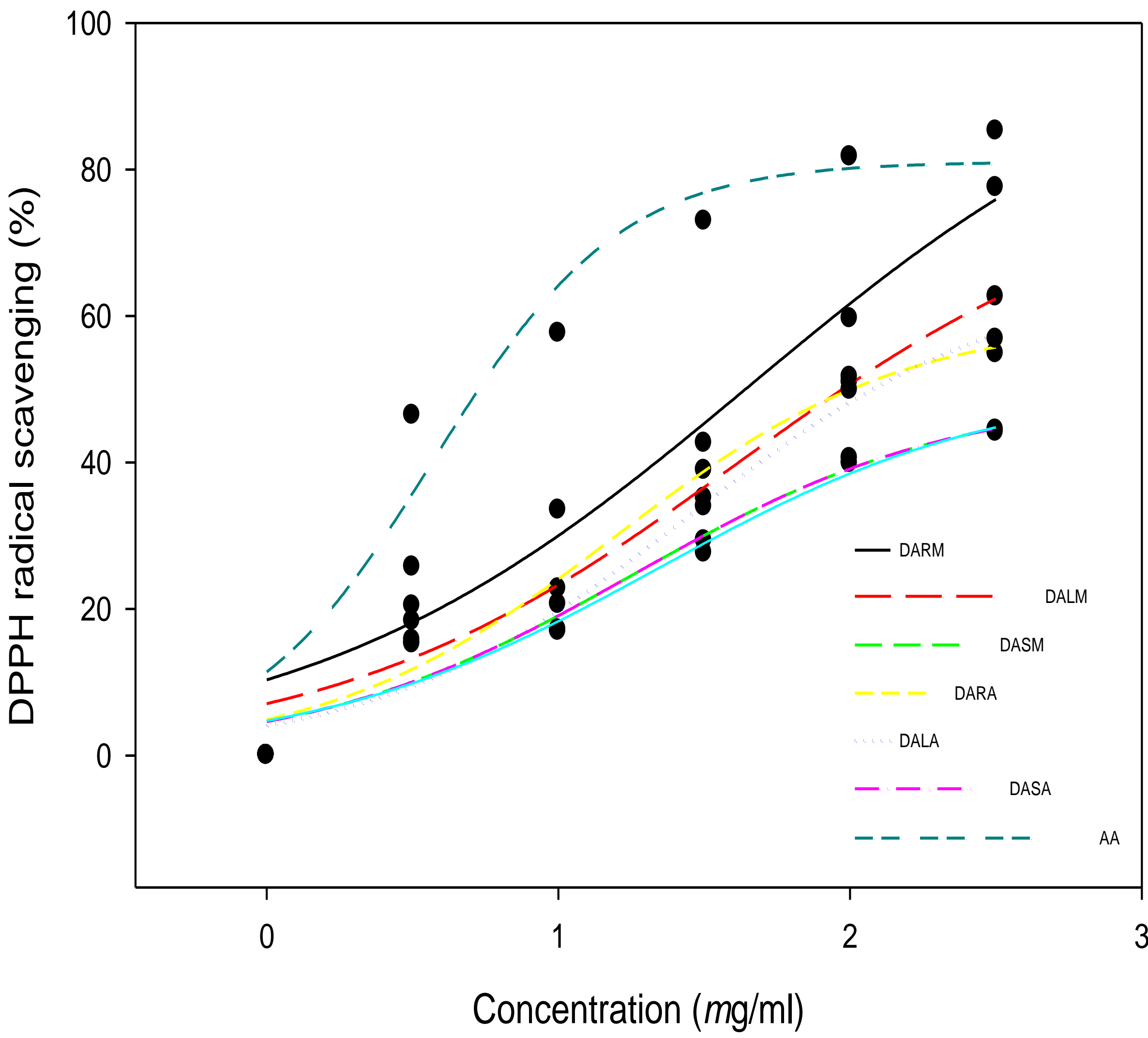

Figure 4.1: Effects of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid (AA) on scavenging of 2,2-diphenyl-1-picryhydrazyl (DPPH) radical

Table 4.7: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanolic extracts of leaves, stem and roots of *C.dependens* and Ascorbic acid standard on DPPH radicals

Extracts	Threshold inhibitory concentration (*IC_{50})	Mathematical Model	Equation	R^2
CDRM	1.81 ± 0.32	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.941
CDLM	1.44 ± 0.14	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.956
CDSM	1.52 ± 0.10	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.975
CDRA	1.10 ± 0.12	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.939
CDLA	1.81 ± 0.32	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.942
CDSA	1.51 ± 0.14	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.966
AA	0.58 ± 0.05	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.934

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.

Key: CDRM = *C. dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C. dependens* stem methanol, **CDRA** = *C. dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous, **AA** = Ascorbic acid

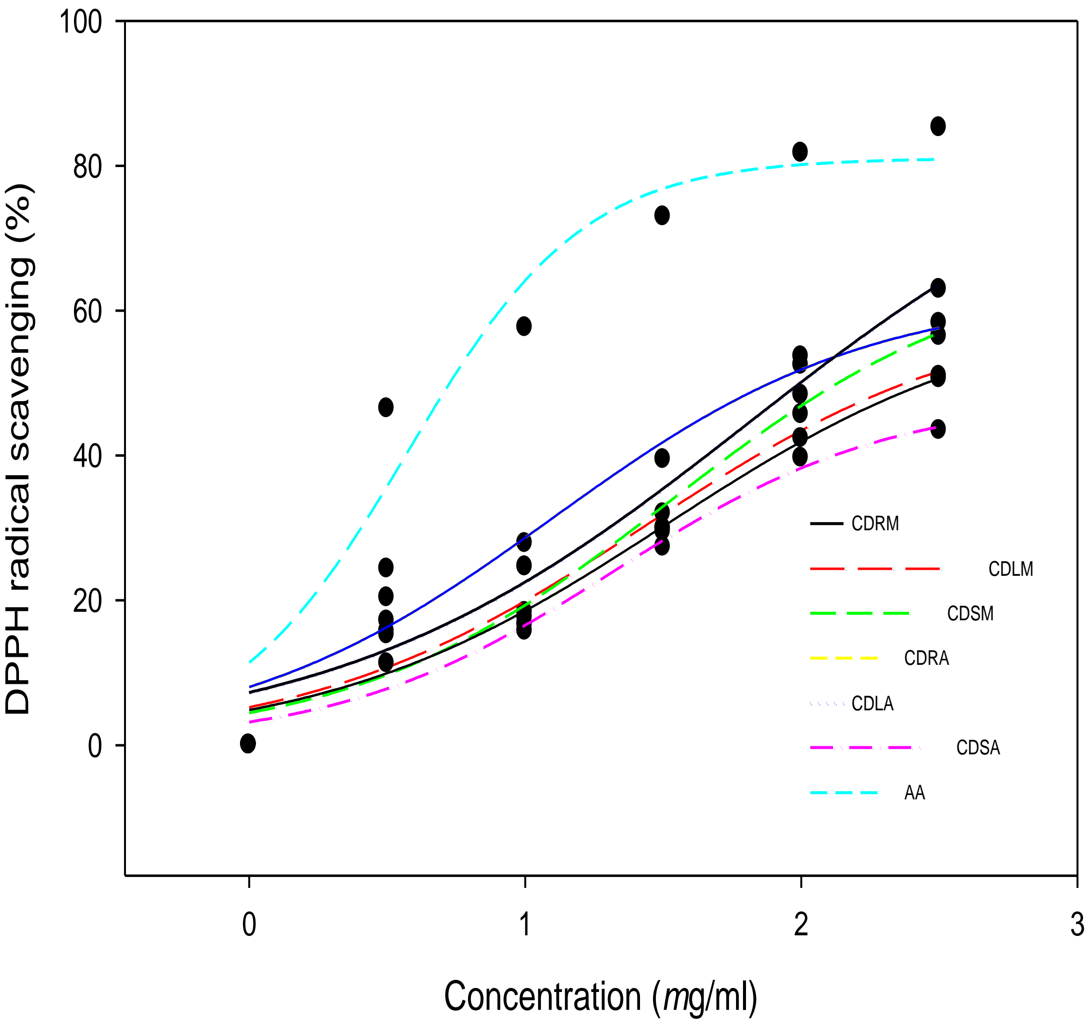

Figure 4.2: Effects of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and ascorbic acid (AA) on scavenging of 2,2-diphenyl-1-picryhydrazyl (DPPH) radical

4.1.3.2 Effects of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens*, *D. arborescens* and Ascorbic acid on Scavenging Nitric Oxide Radical.

Table 4.9 shows that the percentage inhibition of nitric oxide by the extracts was comparable to that recorded for ascorbic acid (standard). Activity of ascorbic acid was higher than those of the extracts against nitric oxide radical, with IC_{50} value of 0.65 ± 0.07 mg/ml. Nitric oxide radical scavenging activities were concentration dependent. Highest activity, with inhibitory concentration of 0.72 ± 0.11 mg/ml was recorded for aqueous of *D.arborescens* root. This activity was however, significantly ($p<0.05$) lower than Ascorbic acid whose IC_{50} was 0.65 ± 0.07 mg/ml. Figure 4.3 shows the nitric oxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid (AA) standard. Results obtained shows that nitric oxide radical scavenging activities of the extracts were sigmoidal. Extracts recorded effective inhibitory effect against nitric oxide radicals when compared to the standard. Mathematical modeling of results are presented on Table 4.8; the threshold Inhibitory concentration (IC_{50}) values were 0.82 ± 0.03, 1.64 ± 0.26, 1.46 ± 0.21, 0.72 ± 0.11, 3.1 ± 0.21, 2.40 ± 0.32 and 0.65 ± 0.07 mg/ml for DARM, DALM, DASM, DARA, DALA, DASA and AA respectively. The results also indicated a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal pattern, model (sigmoid a, b, c) with the equation shown in Table 4.8. IC_{50} values show that nitric oxide radical scavenging activity was in the order AA > DARA > DARM > DASM >DALM > DALA > DASA.

Figure 4.4 shows nitric oxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *C.dependens* and ascorbic acid (AA) standard. Results obtained shows that scavenging activities of methanol extracts were higher than those of aqueous extracts as shown in their IC_{50} values evaluated from the inhibition graph (Fig 4.4). Activities of the extracts

were however significantly ($p<0.05$) lower than that of the standard. Results presented on Figure 4.4 shows that ability of extracts to scavenge nitric oxide, generated *in vitro* by sodium nitroprusside, followed a sigmoid a, b, c model. Mathematical modeling of results is presented on Table 4.9, threshold Inhibitory concentration (IC_{50}) values were 1.16 ± 0.12, 1.10 ± 0.11, 1.25 ± 0.26, 1.32 ± 0.14, 1.62 ± 0.24, 1.36 ± 0.22 and 0.65 ± 0.07 mg/ml for the extracts CDRM, CDLM, CDSM, CDRA, CDLA, CDSA and AA respectively. The R^2 value ranged from 0.89-0.95, indicating a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal pattern, model (sigmoid a, b, c) with the equation shown in Table 4.7. Comparison of evaluated IC_{50} values showed that nitric oxide radical scavenging was in the order AA > CDLM >CDRM > CDSM > CDRA > CDSA > CDLA.

Table 4.8: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* and Ascorbic acid standard on nitric oxide radicals

Extracts	Threshold inhibitory concentration (*IC_{50})	Mathematical Model	Equation	R^2
DARM	0.82 ± 0.03	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.99
DALM	1.64 ± 0.26	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.95
DASM	1.46 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.93
DARA	0.72 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.87
DALA	3.1 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.88
DASA	2.40 ± 0.32	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.94
AA	0.65 ± 0.07	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.91

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.

Key: DARM = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D. arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D. arborescens* leaves aqueous, **DASA** = *D. arborescens* stem aqueous, AA = Ascorbic acid

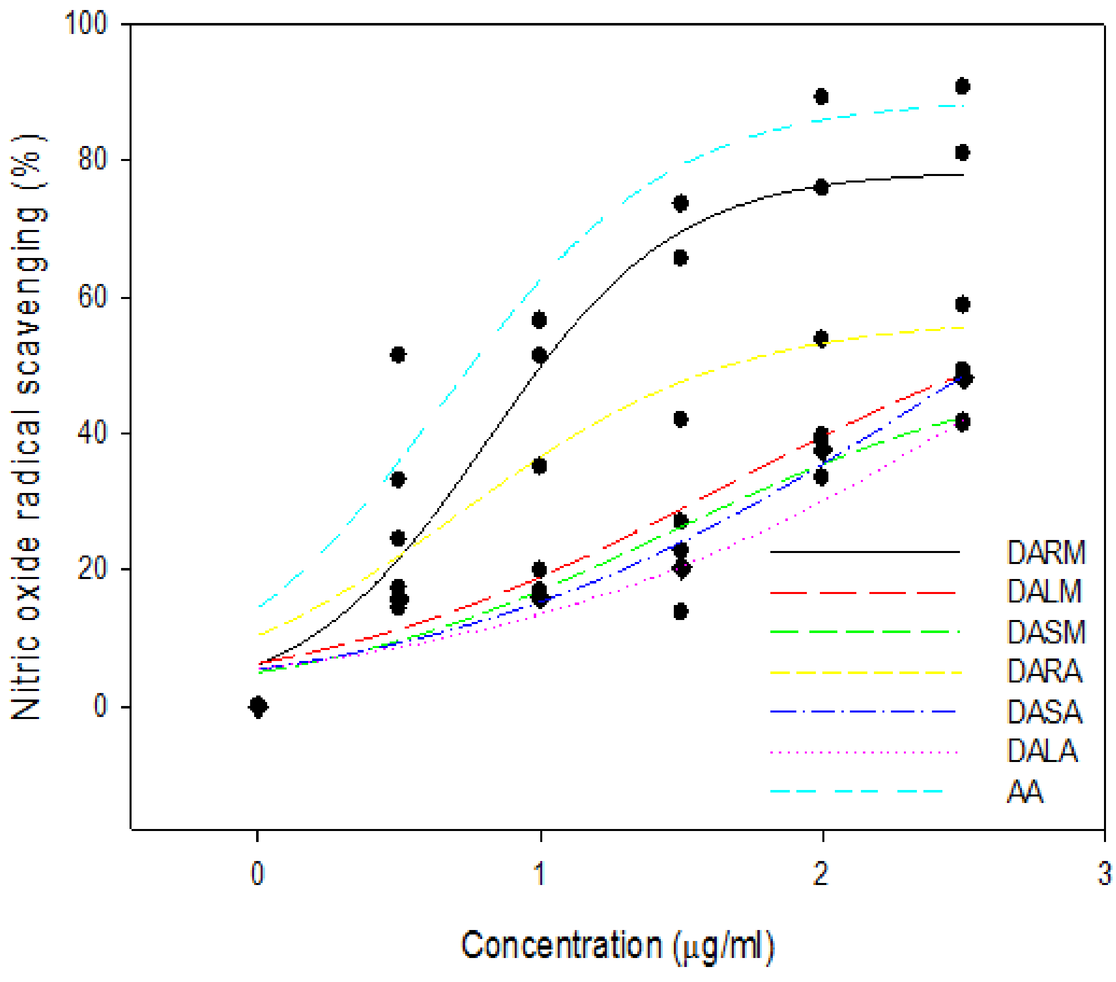

Figure 4.3: Nitric oxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and Ascorbic acid standard

Table 4.9: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanolic extracts of leaves, stem and roots of *C. dependens* and Ascorbic acid standard on nitric oxide radicals

Extracts	Threshold inhibitory concentration (IC_{50})	Mathematical Model	Equation	R^2
CDRM	1.16 ± 0.12	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.95
CDLM	1.10 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.94
CDSM	1.25 ± 0.26	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.89
CDRA	1.32 ± 0.14	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.95
CDLA	1.62 ± 0.24	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.94
CDSA	1.36 ± 0.22	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.92
AA	0.65 ± 0.07	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.91

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.

Key: CDRM = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous, **AA** = Ascorbic acid

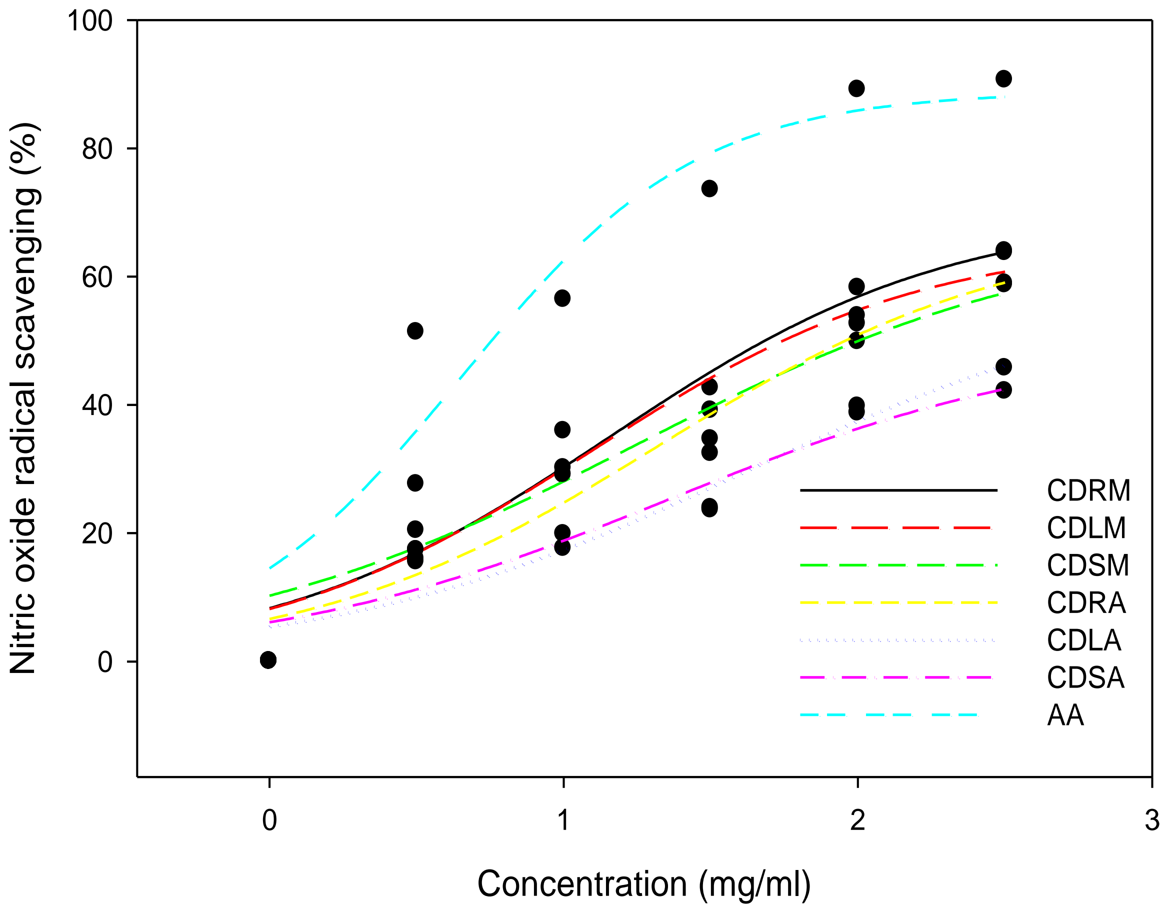

Figure 4.4: Nitric oxide radicals scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and Ascorbic acid standard

4.1.3.3 Effects of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens, D. arborescens* and Ascorbic acid on Scavenging Hydrogen Peroxide (H_2O_2) Radical

Fig 4.5 represents the hydrogen peroxide scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *D.arborescens* with the standard antioxidant (ascorbic acid). Results obtained shows that the extracts inhibited H_2O_2 compared with ascorbic acid. Methanol extract of *D.arborescens* root recorded the highest activity (IC_{50} = 0.42 ± 0.00 mg/ml) at the highest concentration of 2.5 mg/ml. This differed significantly ($p<0.05$) with the standard antioxidant (ascorbic acid) whose IC_{50} was 0.38 ± 0.01 mg/ml at the same concentration.The inhibitory concentration (IC_{50}) values of the extracts were 0.42 ± 0.00, 0.64 ± 0.10, 1.02 ± 0.11, 0.94 ± 0.15, 1.19 ± 0.21, 2.08 ± 0.35 and 0.38 ± 0.01 mg/ml for DARM, DALM, DASM, DARA, DALA, DASA and AA respectively (Table 4.10). IC_{50} values shows that hydrogen peroxide radical scavenging activity was in the order AA > DARM > DALM > DARA > DASM> DALA > DASA. Figure 4.5 shows the hydrogen peroxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid (AA) standard. From results obtained, hydrogen peroxide radical scavenging activities of the extracts were largely sigmoidal; while the standard antioxidant followed a logistic dose-dependent pattern. The extracts also demonstrated effective inhibitory effects against hydrogen peroxide radicals comparable to standard. Mathematical modeling of results indicated a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal model (sigmoid a, b, c) for the extracts, and logistic dose response (a, b, c) for the standard (Table 4.10).

Figure 4.6 shows the hydrogen peroxide radical scavenging activities of *C.dependens* and ascorbic acid (AA) standard. Result obtained also shows that scavenging activities of the extracts were largely sigmoidal, while the standard followed a logistic dose-dependent pattern. Inhibitory effects

of the extracts against hydrogen peroxide radicals were comparable to the standard. Mathematical modeling of results is presented on Table 4.11 with the threshold Inhibitory concentration (IC_{50}) values. Results obtained indicates a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a sigmoidal pattern, model (sigmoid a, b, c) for the extracts and logistic dose response (a, b, c) for standard (Table 4.11). Hydrogen peroxide radical scavenging activity of leaves, stem and roots of *C.dependens* was in the order AA > CDSA > CDRA > CDRM > CDLA > CDSM > CDLM.

Table 4.10: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanol extracts of *D. arborescens* and Ascorbic acid standard on hydrogen peroxide radicals

Extracts	Threshold inhibitory concentration (IC_{50})	Mathematical Model	Equation	R^2
DARM	0.42 ± 0.00	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.999
DALM	0.64 ± 0.10	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.845
DASM	1.02 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.931
DARA	0.94 ± 0.15	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.888
DALA	1.19 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.910
DASA	2.08 ± 0.35	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.920
AA	0.38 ± 0.01	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.999

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.

Key: DARM= *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **AA** = Ascorbic acid

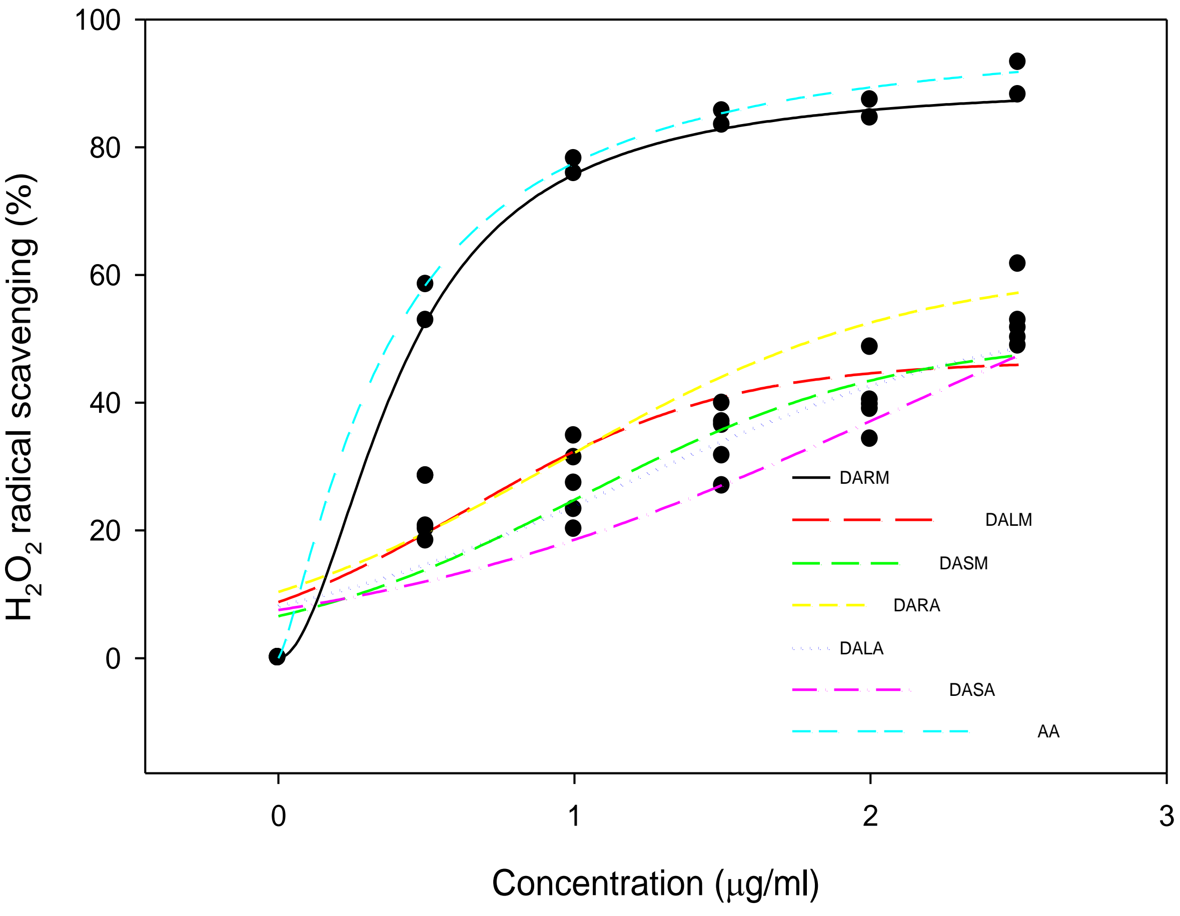

Figure 4.5: Hydrogen peroxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and Ascorbic acid standard

Table 4.11: Threshold Inhibitory concentration (IC_{50}) of crude aqueous and methanol extracts of *C.dependens* and Ascorbic acid standard on hydrogen peroxide radicals

Extracts	Threshold inhibitory concentration (IC_{50})	Mathematical Model	Equation	R^2
CDRM	0.90 ± 0.09	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.931
CDLM	1.17 ± 0.17	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.920
CDSM	1.16 ± 0.15	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.915
CDRA	0.71 ± 0.07	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.915
CDLA	0.93 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.911
CDSA	0.59 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.810
AA	0.38 ± 0.01	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.999

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.

Key: CDRM = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous, **AA** = Ascorbic acid

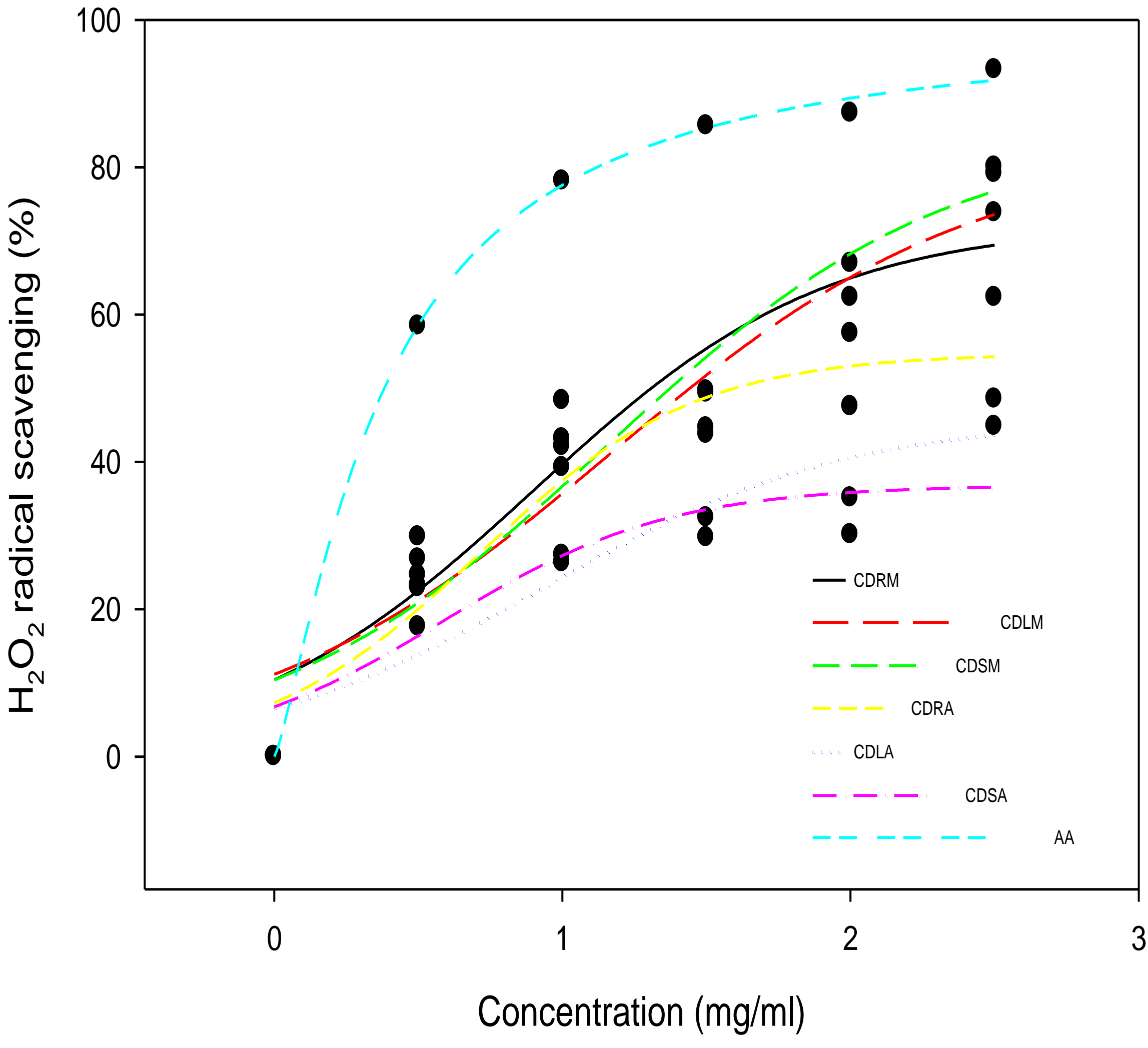

Figure 4.6: Hydrogen peroxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *C.dependens* and Ascorbic acid standard

4.1.3.4 Ferric reducing antioxidant power of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens*, *Dictyandra arborescens* and ascorbic acid

According to Hsu *et al*. (2006), reducing capacity of an extarct serves as a significant indicator of its potential antioxidant. Absorbance of the plant extracts was in a dose-dependent manner as increase in concentration of extracts increased absorbance. Figure 4.7 shows the ferric reducing antioxidant power of crude aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid. Result obtained also shows that ascorbic acid was a more potent reducing agent, with $RP_{0.5}$ of 0.18±0.00 mg/ml, while DARM, DALM, DASM, DARA and DASA had reducing power of 0.30 ± 0.02, 1.38 ± 0.16, 58.31 ± 0.11, 0.84 ± 0.02 and 34.13 ± 0.76 mg/ml respectively (Table 4.10). Results showed that root extracts of *D. arborescens* in aqueous and methanol solvent possessed reducing power activity comparable to the standard as they did not differ significantly ($p<0.05$) from ascorbic acid. Results presented in Figure 4.7 demonstrated a logistic dose-dependent response, reducing effects of extracts on ferric chloride. The data obtained fitted into logistic dose response (a,b,c,d) and Logistic dose response (a,b,c) models, with R^2 values ranging from 0.953 to 0.999 (Table 4.12).

Figure 4.8 shows the Ferric reducing antioxidant power determination results of crude aqueous and methanolic extracts of *C. dependens* and ascorbic acid. Result obtained from the study shows that ascorbic acid was a more potent reducing agent with $RP_{0.5}$=0.18 mg/ml, while CDRM, CDLM, CDSM, CDRA, CDLA and CDSA had reducing power of 0.41 ± 0.05, 0.52 ± 0.07, 2.26 ± 0.11, 0.54 ± 0.05, 0.73 ± 0.04 and 2.67 ± 0.23 mg/ml respectively (Table 4.13). Result shows that the root of *C. dependens* in methanolic solvent possessed reducing power activity comparable to the standard. Results presented in Figure 4.8 demonstrated a logistic dose dependent response

reducing effect of extracts on ferric chloride.by AA, CDRM, and CDLA, CDLM followed a Weibull cumulative (WeibullCum (a,b,c,d)) model, while the reducing power of CDSA, CDSM and CDRA were sigmoidal (Sigmoid a,b,c). R^2 values ranged from 0.953-0.998 (Table 4.13). Ferric reducing antioxidant power was in the order AA > CDRM > CDLM >CDRA > CDLA > CDSM > CDSA.

Table 4.12: Ferric reducing antioxidant power (FRAP) of crude aqueous and methanol extracts of leaves, stem and roots of *Dictyandra arborescens* and ascorbic acid

Extracts	Reducing power ($RP_{0.5}$)	Mathematical Model	Equation	R^2
DARM	0.30 ± 0.02	Logistic dose response (a,b,c,d)	$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^{d}}$	0.992
DALM	1.38 ± 0.16	Logistic dose response (a,b,c,d)	$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^{d}}$	0.999
DASM	2.16 ± 0.05	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^{c}}$	0.999
DARA	0.84 ± 0.02	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^{c}}$	0.958
DALA	1.99 ± 0.09	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.994
DASA	2.82 ± 0.48	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^{c}}$	0.994
AA	0.18 ± 0.00	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^{c}}$	0.953

Key: DARM= *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **AA** = Ascorbic acid

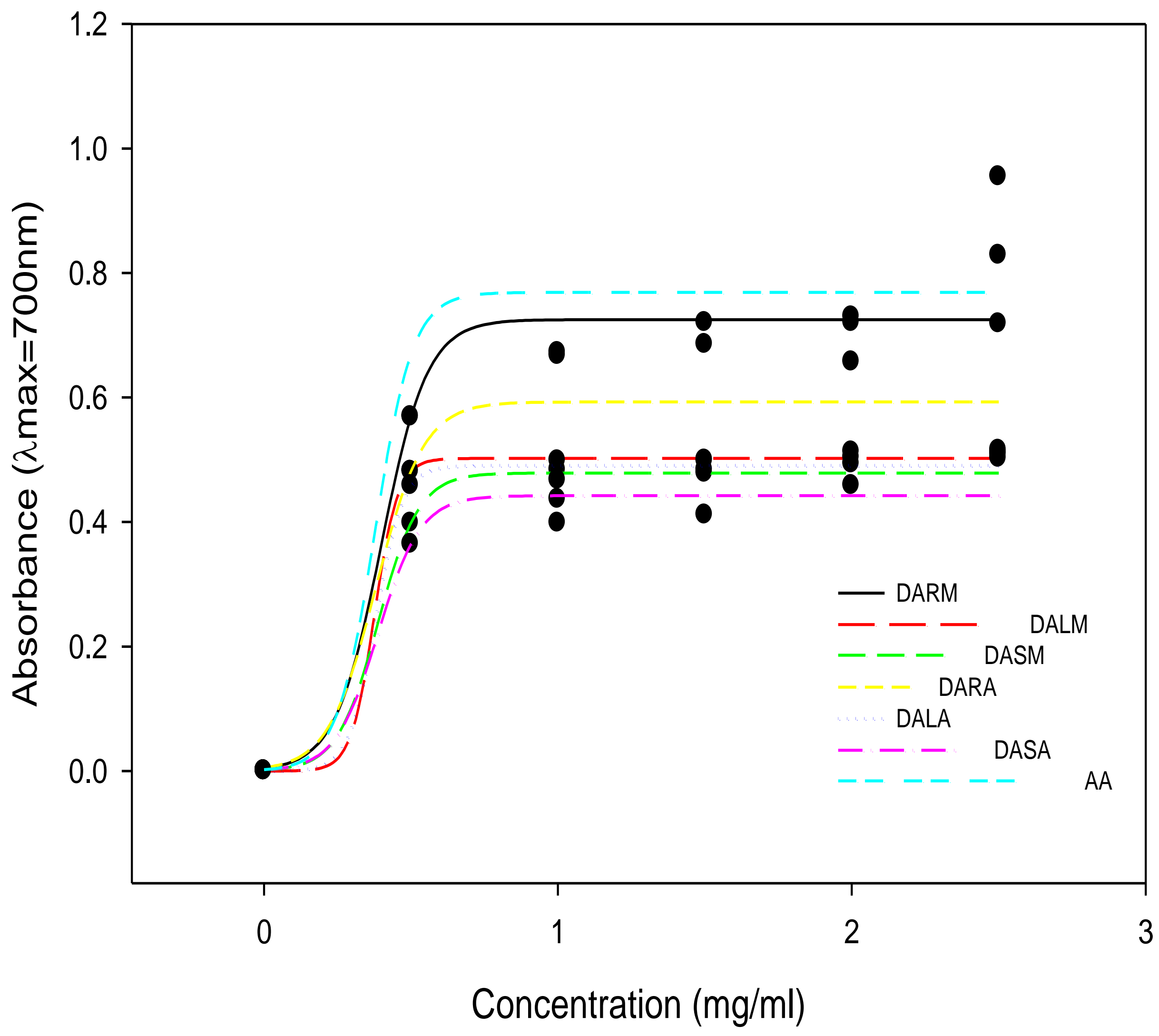

Figure 4.7: Ferric reducing antioxidant power (FRAP) of crude aqueous and methanolic extracts of leaves, stem and roots of *Dictyandra arborescens* and ascorbic acid

Table 4.13: Ferric reducing antioxidant power (FRAP) of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and ascorbic acid

Extracts	Reducing power ($RP_{0.5}$)	Mathematical Model	Equation	R^2
CDRM	0.41 ± 0.05	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.992
CDLM	0.52 ± 0.07	WeibullCum (a,b,c,d)	$y = a\left[1 - exp\left[-\left[\frac{x + c(In\ 2)^{1/d} - b}{c}\right]^d\right]\right]$	0.998
CDSM	2.26 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.973
CDRA	0.54 ± 0.05	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.965
CDLA	0.73 ± 0.04	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.997
CDSA	2.67 ± 0.23	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x - x_0}{b}\right)\right)}$	0.954
AA	0.18 ± 0.00	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.953

Key: CDRM = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous, **AA** = Ascorbic acid

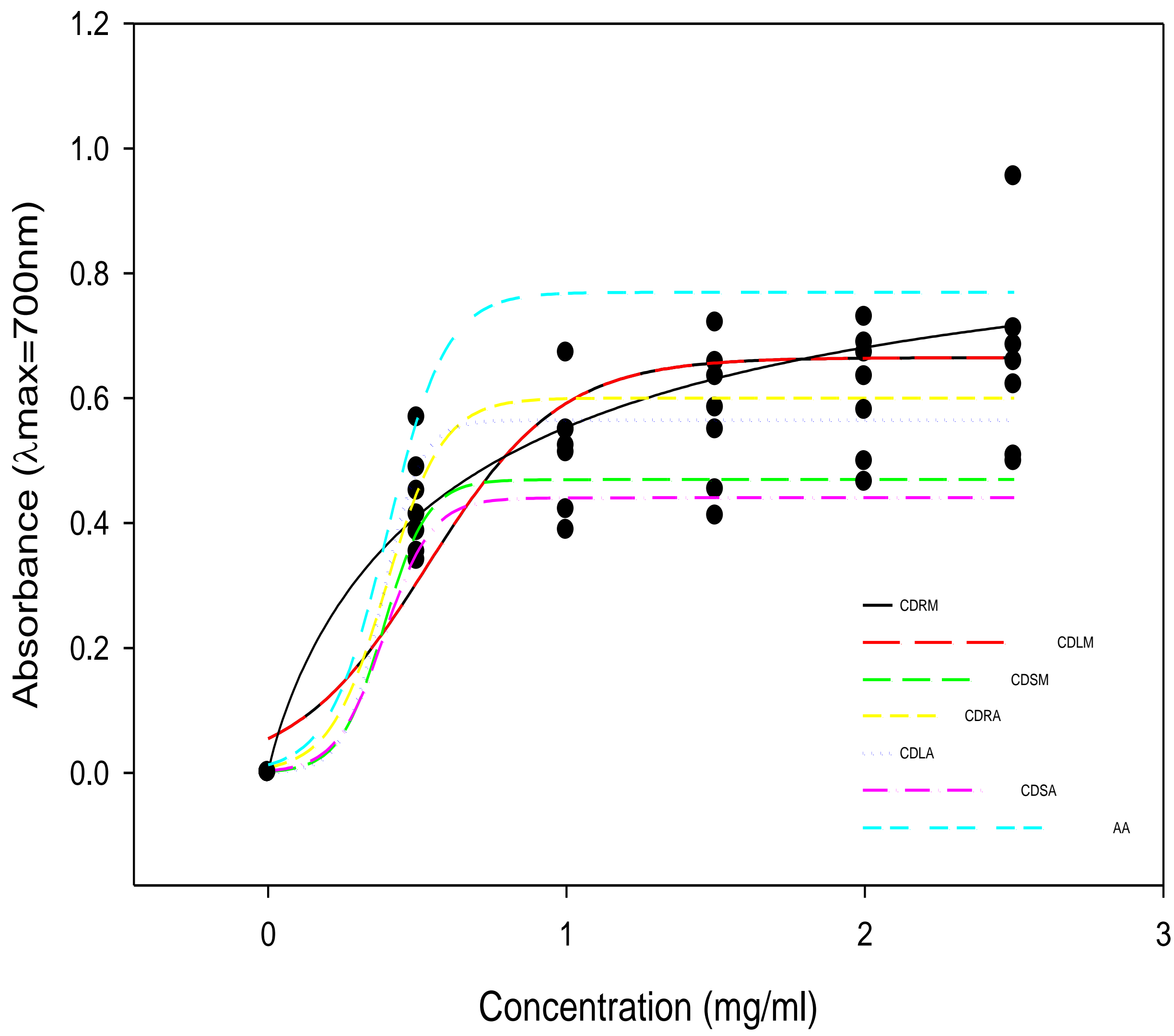

Figure 4.8: Ferric reducing antioxidant power (FRAP) of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and ascorbic acid

4.1.3.5 Total antioxidant capacity (TAC) of crude aqueous and methanolic extracts of leaves, stem, and roots of *Dictyandra arborescens* and *Chasmanthera dependens*

Table 4.14 shows results of Total antioxidant capacity (TAC) of crude aqueous and methanolic extracts of leaves, stem and roots of *Dictyandra arborescens* and *Chasmanthera dependens* expressed as mg AA / g extract. The TAC of the extracts were 2352.94 ± 164.87, 1050.42 ± 20.61, 1046.22 ± 37.78, 1050.42 ± 13.74, 920.17 ± 17.17 and 201.68 ± 13.74 mg AA /g extract for DARM, DALM, DASM, DARA, DALA and DASA respectively; while TAC values of CDRM, CDLM, CDSM, CDRA, CDLA and CDSA were, 1865.55 ± 27.48, 2105.04 ± 92.74, 1970.59 ± 17.17, 1731.09 ± 13.74, 1701.68 ± 10.31 and 1647.06 ± 13.74 mg AA /g extract respectively (Table 4.14). Results shows that methanol extract of *D. arborescens* root had the highest TAC followed by methanol extract of the leaves of *C. dependens*. The least TAC value was obtained from aqueous extract of *D. arborescens* stem.

Results obtained in this study shows that extracts of the leaves, stems and roots of the *D.arborescens* and *C.dependens* possess good antioxidants properties.

Table 4.14 Total antioxidant content (TAC) of crude aqueous and methanolic extracts of *Dictyandra arborescens* and *Chasmanthera dependens* expressed as Ascorbic acid equivalent (AA) / g extract

Extract	TAC (mg AA /g extract)
DARM	2352.94 ± 164.87
DALM	1050.42 ± 20.61
DASM	1046.22 ± 37.78
DARA	1050.42 ± 13.74
DALA	920.17 ± 17.17
DASA	201.68 ± 13.74
CDRM	1865.55 ± 27.48
CDLM	2105.04 ± 92.74
CDSM	1970.59 ± 17.17
CDRA	1731.09 ± 13.74
CDLA	1701.68 ± 10.31
CDSA	1647.06 ± 13.74

Values are Mean ± standard error of mean (SEM) of 3 determinations

Key: DARM= *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** =*D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** =*C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** =*C. dependens* leaves aqueous, **CDSA** =*C.dependens* stem aqueous.

4.1.4 Acute toxicity test (LD_{50})

In the first phase of the test, administration of 10, 100 and 1000mg/kg body weight of aqueous and methanol extracts of the leaves, stem and roots of both plants did not induce any sign of toxicity and no death was recorded for the different extracts of the two plants (Tables 4.15 and 4.16). In the second phase of the test, which was based on the result of the first phase, no sign of toxicity or death was recorded in the mice even at high doses of 1600, 2900 and 5000mg/kg body weight for the roots, stem and leaves of *Dictyandra arborescens* as well as roots and leaves of *Chasmanthera dependens* (Tables 4.17 and 4.18). Signs of toxicity and death were however recorded in aqueous and methanolic extracts of the stem of *Chasmanthera dependens*, at doses of 2900 and 5000mg/kg body weight of the extracts respectively. This indicated that extracts of the leaves, stem and roots of *Dictyandra arborescens,* as well as extracts of the roots and leaves of *Chasmanthera dependens* were safe, even at a very high dose of 5000mg/kg body weight (Table 4.17). The highest tolerated dose for aqueous and methanolic extracts of the stem of *Chasmanthera dependens* was 3808mg/kg body weight of the extract (Table 4.18).

Table 4.15: Acute toxicity test of the aqueous and methanolic extracts of the roots, leaves, and stem of *Dictyandra arborescens* at doses of 10, 100 and 1000 mg/kg body weight.

Extracts	Number of animals per group	Dose of extract/ Weight (g) of mice (10 mg/kg)	Signs of toxicity	Number of animals that survived
DARM	3	22g	x	3/3
DARA	3	20	x	3/3
DALM	3	18	x	3/3
DALA	3	25	x	3/3
DASM	3	24	x	3/3
DASA	3	23	x	3/3
		100 mg/kg		
DARM	3	24	x	3/3
DARA	3	20	x	3/3
DALM	3	25	x	3/3
DALA	3	19	x	3/3
DASM	3	21	x	3/3
DASA	3	24	x	3/3
		1000 mg/kg		
DARM	3	21	x	3/3
DARA	3	19	x	3/3
DALM	3	23	x	3/3
DALA	3	22	x	3/3
DASM	3	19	x	3/3
DASA	3	21	x	3/3

Key: DARM= *D. arborescens* root methanol, **DARA** = *D.arborescens* root aqueous, **DALM** = *D.arborescens* leaves methanol, **DALA** = *D.arborescens* leaves aqueous, **DASM** = *D.arborescens* stem methanol, **DASA** = *D.arborescens* stem aqueous, **x = no sign of toxicity**

Table 4.16: Acute toxicity test of the aqueous and methanolic extracts of the roots, leaves, and stem of *Chasmanthera dependens* at doses of 10, 100 and 1000 mg/kg body weight.

Extracts	Number of animals per group	Dose of extract/ weight (g) of mice (10 mg/kg)	Signs of toxicity	Number of animals that survived
CDRM	3	21	x	3/3
CDRA	3	23	x	3/3
CDLM	3	20	x	3/3
CDLA	3	25	x	3/3
CDSM	3	25	x	3/3
CDSA	3	21	x	3/3
		100 mg/kg		
CDRM	3	23	x	3/3
CDRA	3	22	x	3/3
CDLM	3	24	x	3/3
CDLA	3	20	x	3/3
CDSM	3	19	X	3/3
CDSA	3	22	x	3/3
		1000 mg/kg		
CDRM	3	20	x	3/3
CDRA	3	22	x	3/3
CDLM	3	19	x	3/3
CDLA	3	18	x	3/3
CDSM	3	22	x	3/3
CDSA	3	24	x	3/3

Key: CDRM = *C.dependens* root methanol, **CDRA** = *C.dependens* root aqueous, **CDLM** = *C.dependens* leaves methanol, **CDLA** = *C. dependens* leaves aqueous, **CDSM** = *C.dependens* stem methanol, **CDSA** = *C.dependens* stem aqueous, **x =** no sign of toxicity

Table 4.17: Acute toxicity test (LD_{50}) of the aqueous and methanolic extracts of the roots, leaves, and stem of *Dictyandra arborescens* at high doses of 1600, 2900 and 5000 mg/kg body weight.

Extracts	Number of animals per group	Dose of extract/ Weight (g) of mice	Signs of toxicity	Number of animals that survived
		1600 mg/kg		
DARM	3	19	x	3/3
DARA	3	22	x	3/3
DALM	3	20	x	3/3
DALA	3	18	x	3/3
DASM	3	20	x	3/3
DASA	3	20	x	3/3
		2900 mg/kg		
DARM	3	20	x	3/3
DARA	3	21	x	3/3
DALM	3	21	x	3/3
DALA	3	21	x	3/3
DASM	3	23	x	3/3
DASA	3	22	x	3/3
		5000 mg/kg		
DARM	3	24	x	3/3
DARA	3	19	x	3/3
DALM	3	23	x	3/3
DALA	3	20	x	3/3
DASM	3	22	x	3/3
DASA	3	24	x	3/3

Key: DARM= *D. arborescens* root methanol, **DARA** = *D.arborescens* root aqueous, **DALM** = *D.arborescens* leaves methanol, **DALA** = *D.arborescens* leaves aqueous, **DASM** = *D.arborescens* stem methanol, **DASA** = *D.arborescens* stem aqueous, **x = no sign of toxicity**

Table 4.18: Acute toxicity test (LD_{50}) of the aqueous and methanolic extracts of the roots, leaves, and stem of *Chasmanthera dependens* at high doses of 1600, 2900 and 5000mg/kg body weight.

Plant sample	Number of animals per group	Dose of extract/ Weight(g) of mice	Signs of toxicity	Number of animals that survived
		1600 mg/kg		
CDRM	3	24	x	3/3
CDRA	3	20	x	3/3
CDLM	3	21	x	3/3
CDLA	3	22	x	3/3
CDSM	3	23	x	3/3
CDSA	3	18	x	3/3
		2900 mg/kg		
CDRM	3	20	x	3/3
CDRA	3	21	x	3/3
CDLM	3	18	x	3/3
CDLA	3	21	x	3/3
CDSM	3	24	1	2/3
CDSA	3	22	1	2/3
		5000 mg/kg		
CDRM	3	23	x	3/3
CDRA	3	20	x	3/3
CDLM	3	19	x	3/3
CDLA	3	20	x	3/3
CDSM	3	24	1	2/3
CDSA	3	21	2	1/3

Key: CDRM = *C.dependens* root methanol, **CDRA** = *C.dependens* root aqueous, **CDLM** = *C.dependens* leaves methanol, **CDLA** = *C. dependens* leaves aqueous, **CDSM** = *C.dependens* stem methanol, **CDSA** = *C.dependens* stem aqueous, **x =** no sign of toxicity

4.1.5 Sub-Acute Toxicity Studies

In the sub-acute toxicity study, oral administration of aqueous and methanol extracts of the leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* did not induce any signs of adverse reactions and there were no changes in the general behaviour of the experimental animals during the 14 days of administration of 200 mg/kg body weight of the extracts. Increase in body weight of the animals was recorded in all the groups, as well as the normal and standard control groups (Table 4.19). No mortality was recorded throughout the period of testing.

Table 4.19: Effects of administration of Aqueous and Methanol Extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* on Body Weight of Albino Rats after 14 days

Groups/Extracts	Initial body weight (g)	Final body weight (g)	Weight gain (%)
NC	85.00	98.17±1.30	15.49
STD.C	90.03±0.08	101.17±0.54	12.37
DARM	95.08±0.09	107.0±0.68	12.53
DALM	100.00	109.17±0.61	9.17
DASM	85.08±0.07	94.72±0.62	11.33
DARA	90.03±0.07	100.57±0.89	11.71
DALA	95.02±0.05	103.17±0.45	8.58
DASA	100.05±0.09	108.12±0.76	8.07
CDRM	85.08±0.05	95.2±0.87	11.89
CDLM	90.05±0.04	99.78±0.67	10.81
CDSM	95.04±0.06	102.22±0.67	7.55
CDRA	100.02±0.05	107.55±0.58	7.53
CDLA	85.04±0.02	95.45±0.47	12.24
CDSA	90.06±0.03	100.75±0.98	11.87

Values are mean±SEM

Key: NC = Normal control, **STD. C =** Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous

4.1.6 Heamatological indices

Results obtained in this study shows that heamatological parameters in the albino rats (PCV, RBC, WBC, Hb, MCH, Neutrophils, Lymphocytes, and MCHC) significantly increased ($p<0.05$) on administration of 200 mg/kg body weight of aqueous and methanol extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* (Fig 4.9 - 4.16). For packed cell volume (PCV), results for the standard antimalarial drug and methanol extract of *D.arborescens* root did not differ significantly ($p<0.05$) from the normal control (Fig 4.9). For red blood cells (RBCs), values obtained from the extracts did not differ significantly ($p<0.05$) from the control (Fig 4.10). This also applied for the white blood cells (WBCs) and heamoglobin (Hb), (Fig 4.11 and 4.12). For MCH, data obtained for aqueous and methanol extract of *C.dependens* root and aqueous extract of *C.dependens* stem differed from the normal and standard control ($p<0.05$). Although results obtained for some extracts differed from the normal and standard control for neutrophils, lymphocytes and mean corpuscular haemoglobin concentration, they did not reduce these parameters. This gives an indication that these extracts are not hepatotoxic.

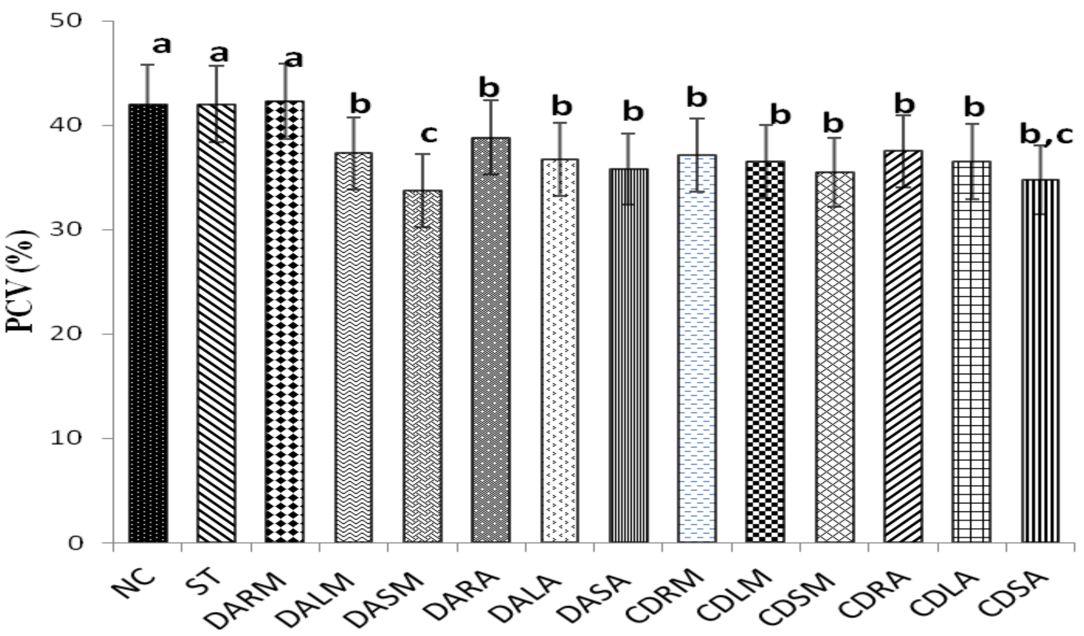

Figure 4.9: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Packed cell volume (PCV) of albino rats

Key: NC = Normal control, ST=Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = D.arborescens leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** =*C.dependens* stem aqueous.

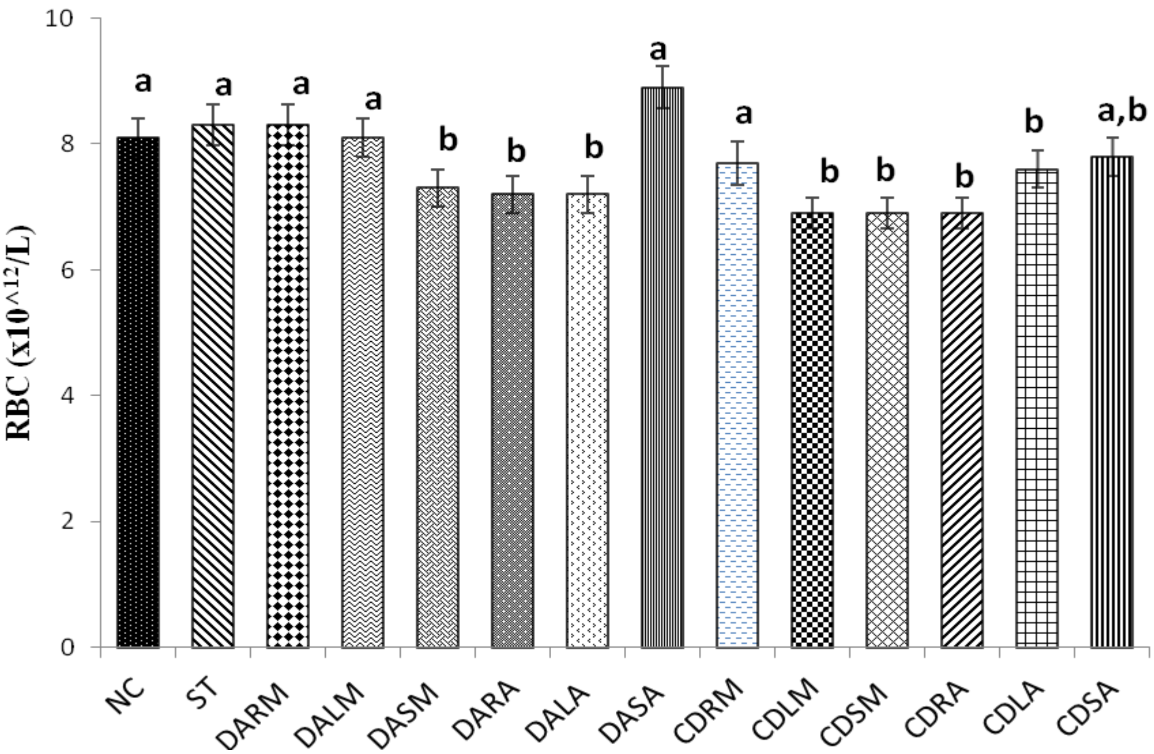

Figure 4.10: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Red blood cells (RBCs) of albino rats

Key: **NC** = Normal control, ST = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = D.arborescens leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

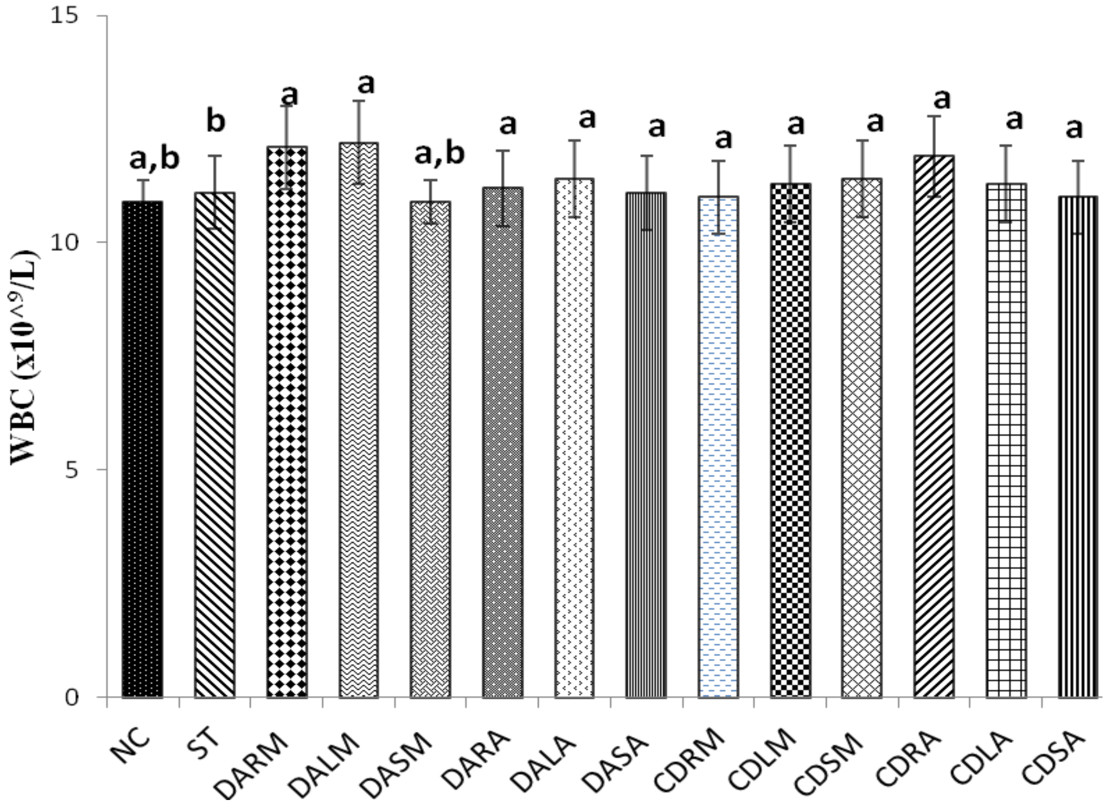

Figure 4.11: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* and *C. dependens* on White blood cells (WBCs) of albino rats

Key: NC = Normal control, ST = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D. arborescens* leaves methanol, **DASM** = *D. arborescens* stem methanol, **DARA** = *D. arborescens* root aqueous, **DALA** = *D. arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C. dependens* root methanol, **CDLM** = *C. dependens* leaves methanol, **CDSM** = *C. dependens* stem methanol, **CDRA** = *C. dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C. dependens* stem aqueous.

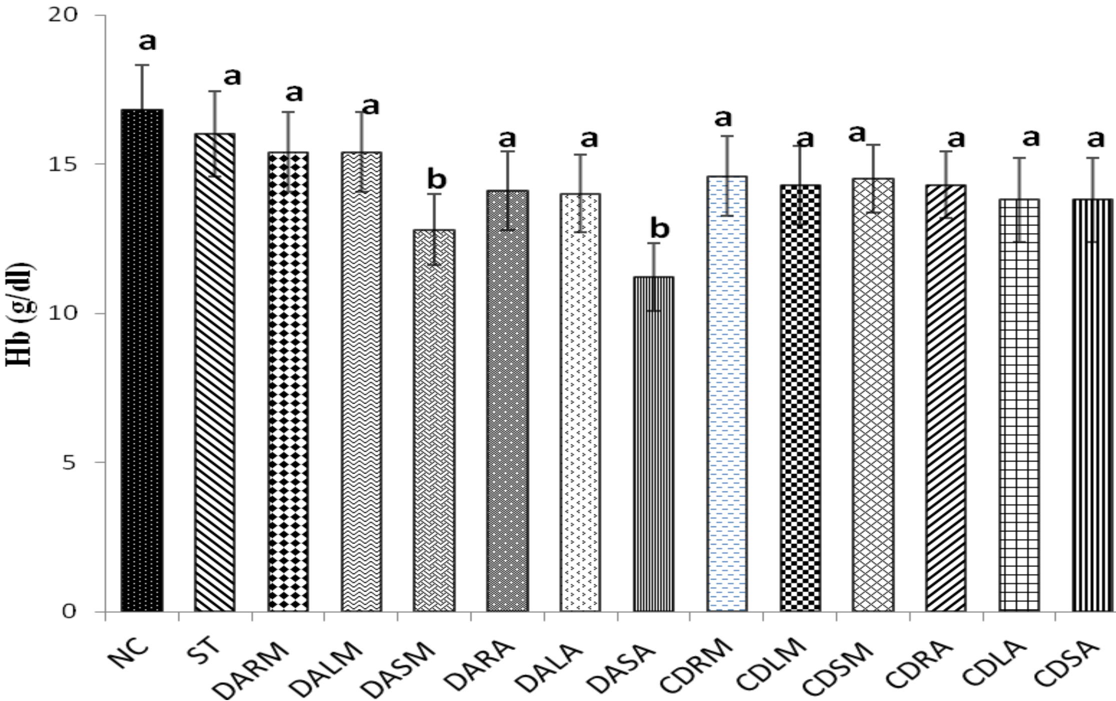

Figure 4.12: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Haemoglobin (Hb) of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

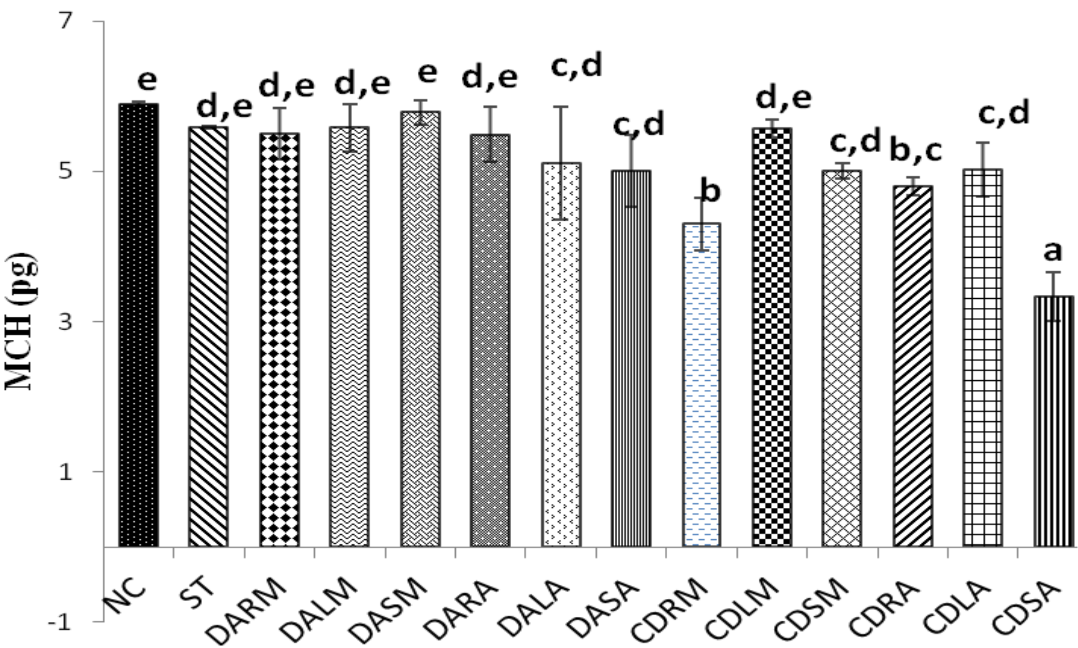

Figure 4.13: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on mean corpuscular haemoglobin (MCH) of albino rats

Key: **NC** = Normal control, ST = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

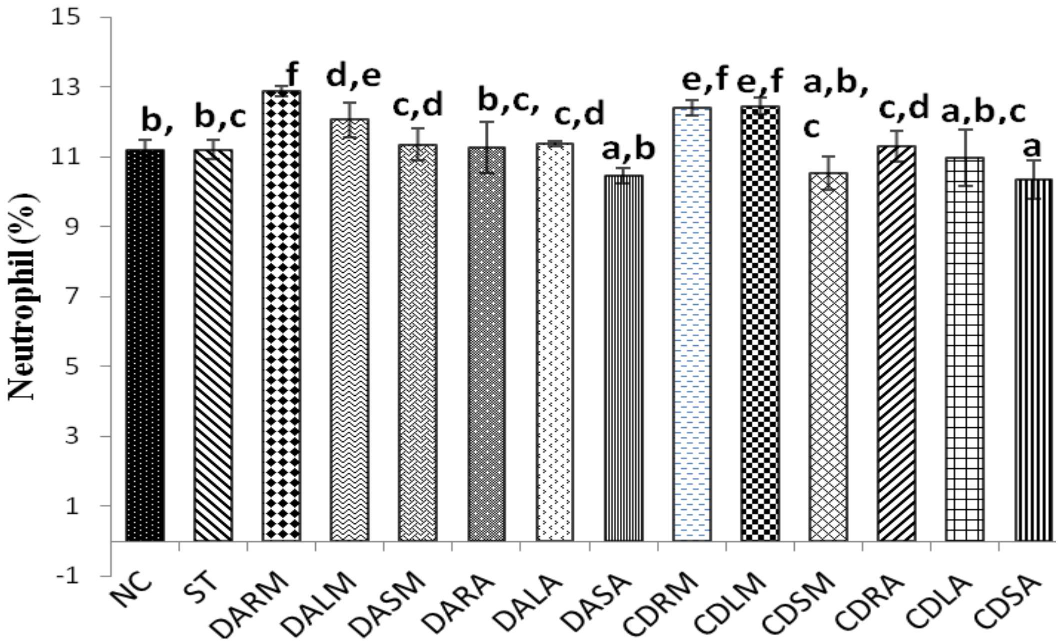

Figure 4.14: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Neutrophils of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

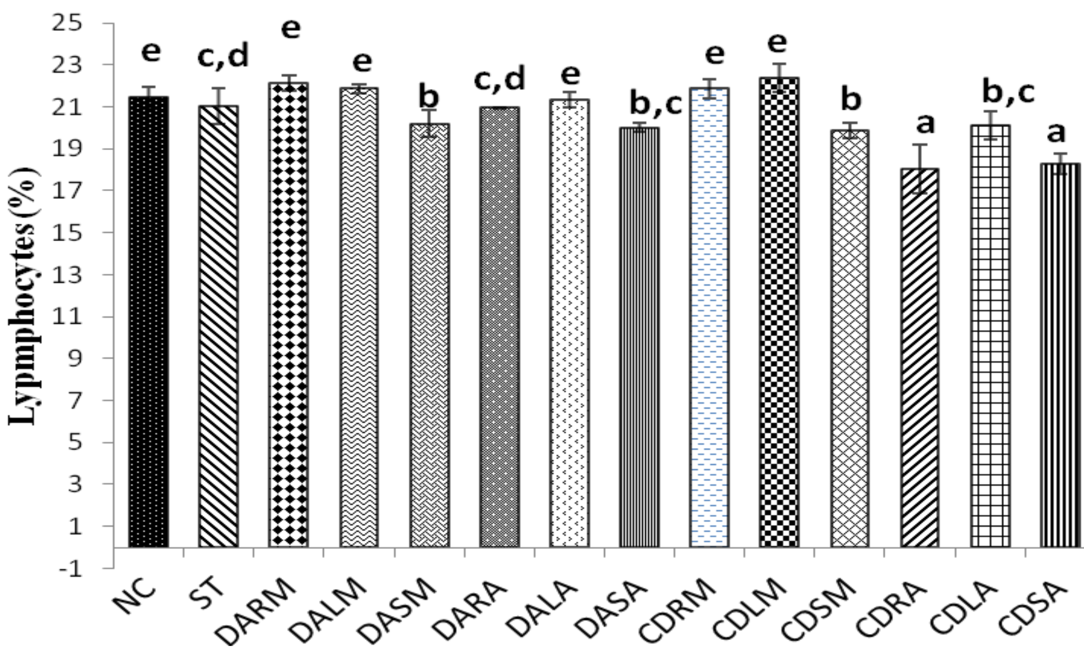

Figure 4.15: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Lymphocytes of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

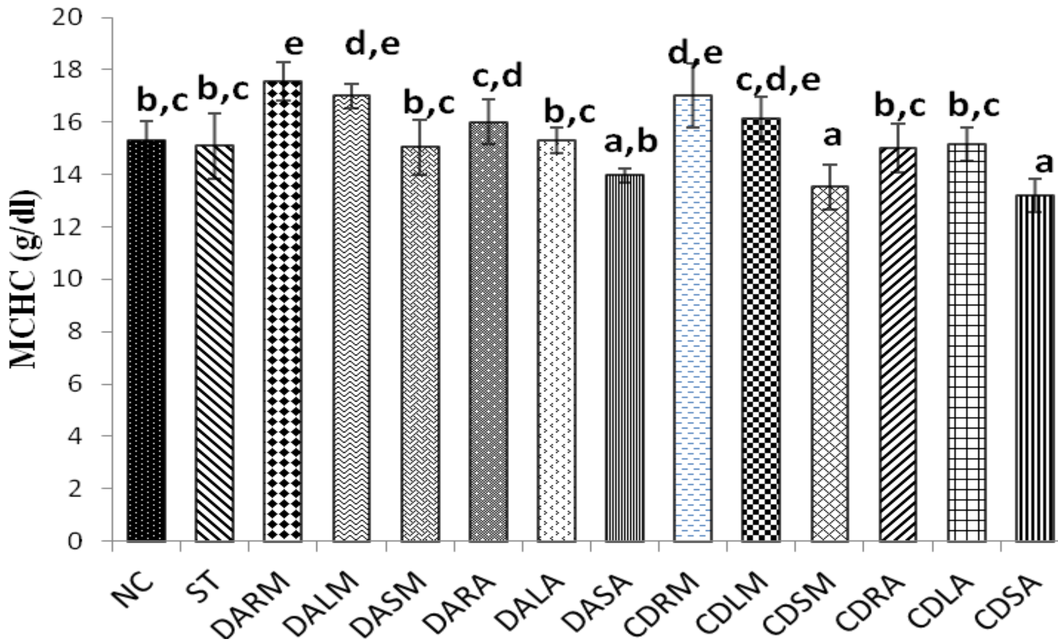

Figure 4.16: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on mean corpuscular haemoglobin concentration (MCHC) of albino rats

Key: NC = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

4.1.7 Biochemical Parameters

Results of the biochemical parameters show that administration of 200 mg/kg body weight of aqueous and methanolic extracts of leaves, stem and roots of the two plants under study did not increase levels of the liver function enzymes (AST, ALT and ALP), serum total protein nor albumin.

For Aspartate transaminase (AST), values obtained for methanol extract of *C.dependens* root (CDRM) and aqueous extract of *C.dependens* leaves (CDLA) differed significantly ($p<0.05$) from the normal and standard control (Fig 4.17). Other extracts did not differ significantly ($p<0.05$) from the normal and standard controls. For Alanine transaminase (ALT), all the extracts did not differ significantly ($p<0.05$) from the normal and standard control (Fig 4.18). For alkaline phosphatase (ALP), results obtained for aqueous extract of *D.arborescens* leaves (DALA) and methanol extract of *C.dependens* leaves (CDLM) differed significantly ($p<0.05$) from the normal and standard control (Fig 4.19). Other extracts did not differ from the normal and standard controls. For serum albumin and total protein, some of the extracts differed significantly ($p<0.05$) from the normal and standard control (Fig 4.20 and Fig. 4.21). However, they did not elevate these biochemical parameters. Results obtained therefore indicate that aqueous and methanol extracts of the leaves, stem and roots of these two plants had no hepatotoxic effects on the experimemtal animals.

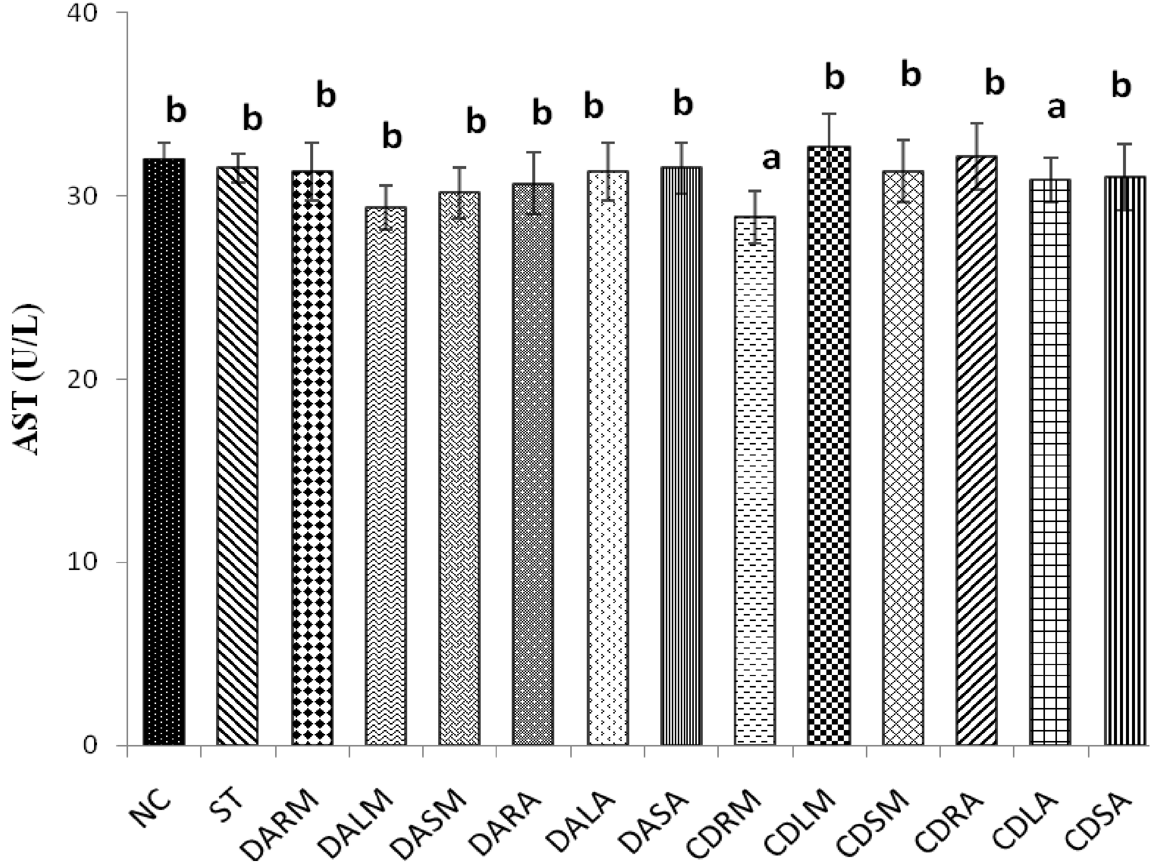

Figure 4.17: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Aspartate transaminase (AST) of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

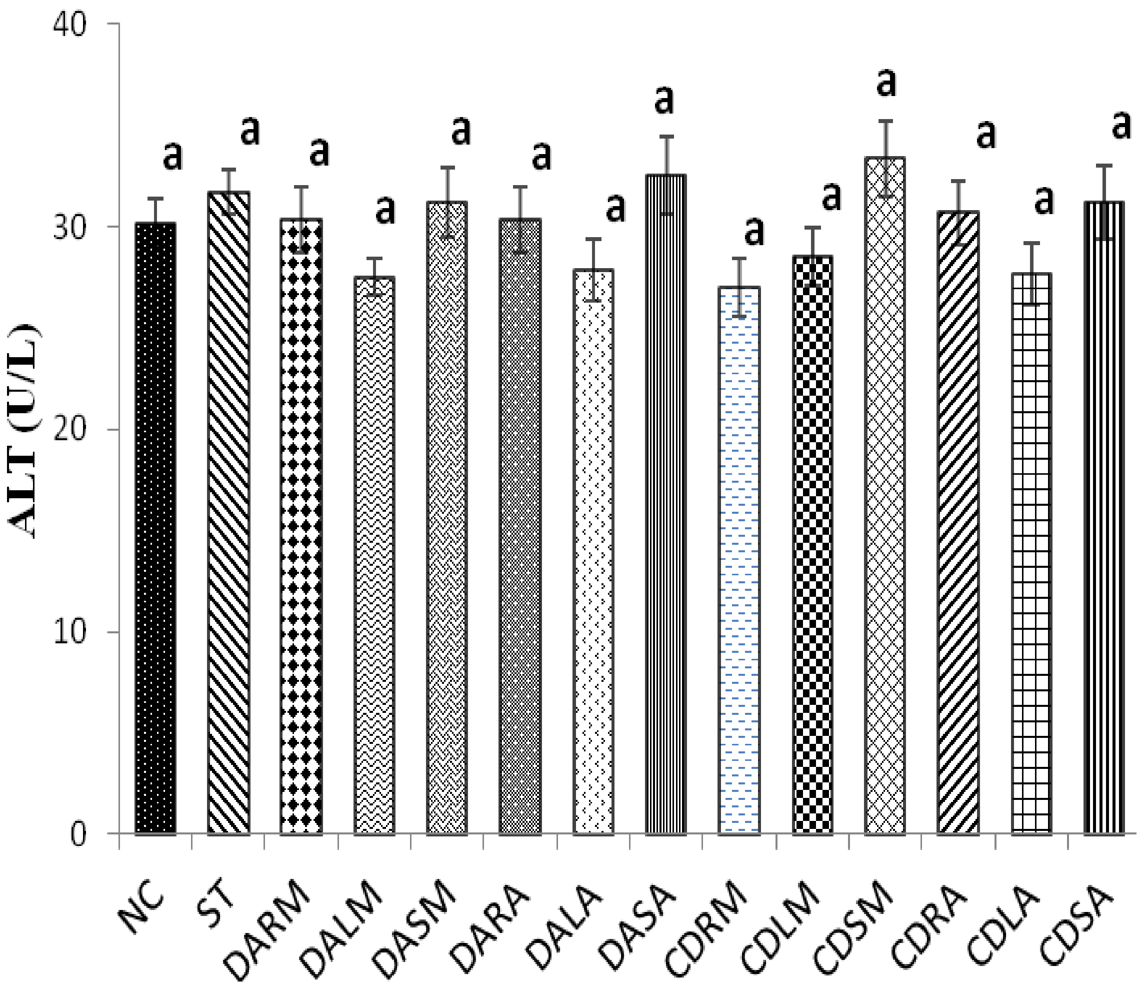

Figure 4.18: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Alanine transaminase (ALT) of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

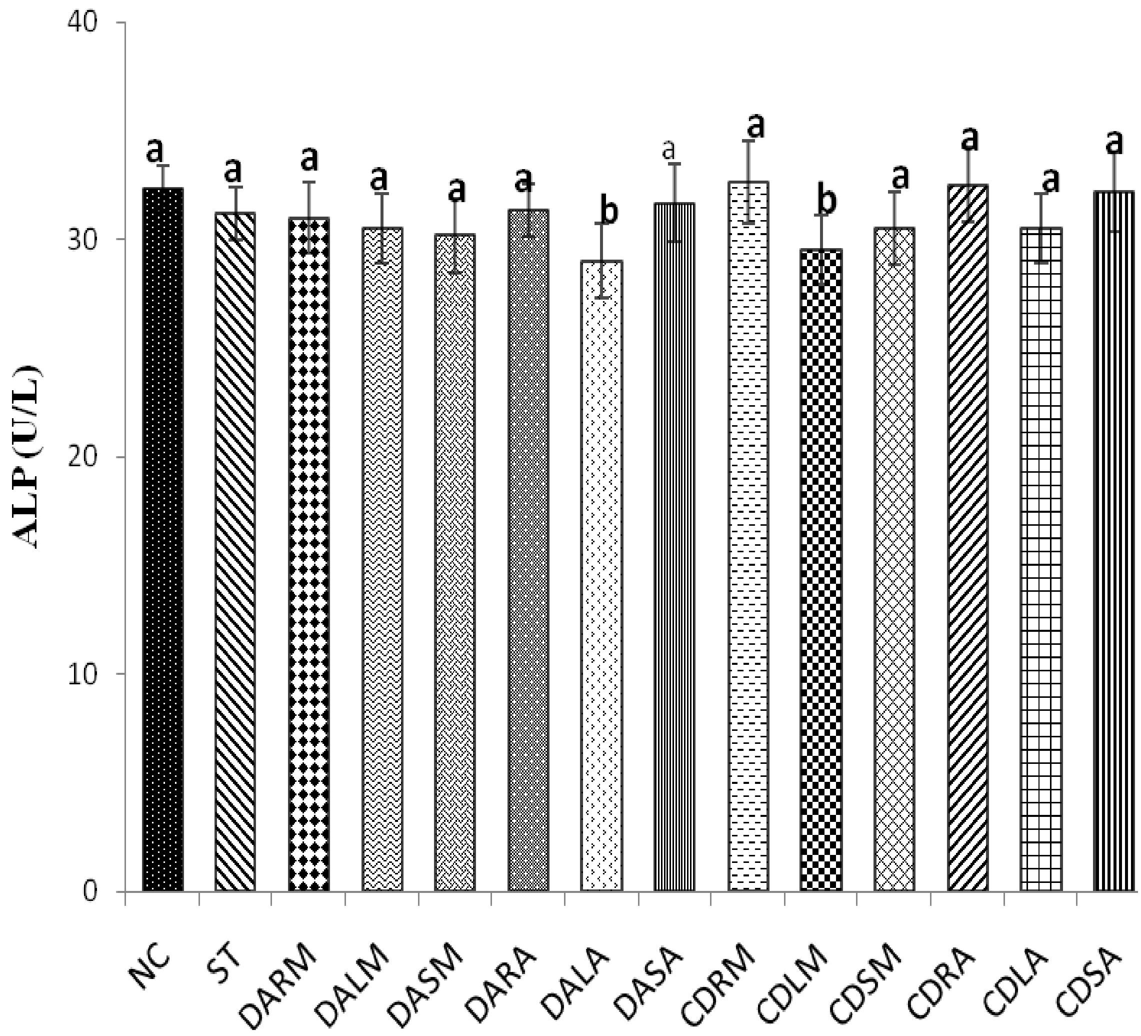

Figure 4.19: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on Alkaline phosphatase (ALP) of albino rats

Key: **NC** = Normal control, **ST** = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous

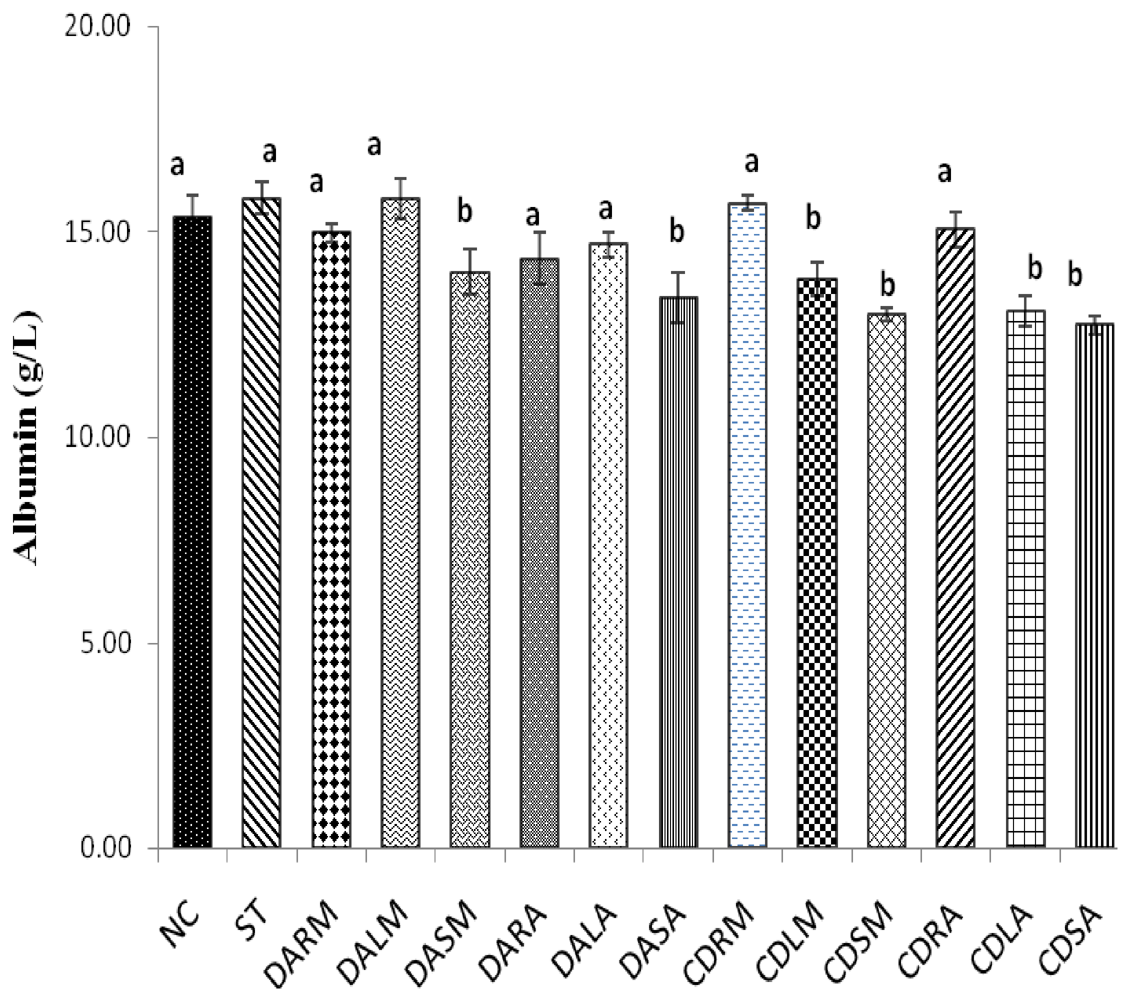

Figure 4.20: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on serum Albumin of albino rats

Key: NC = Normal control, ST = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

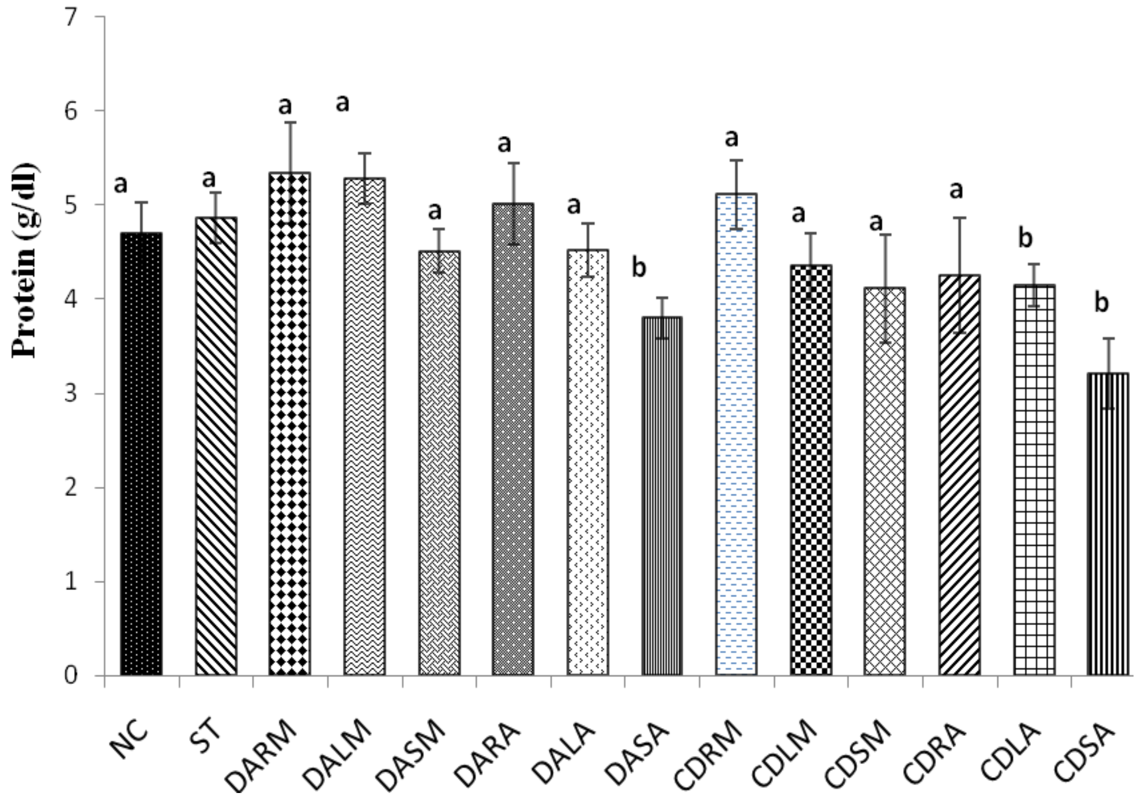

Figure 4.21: Effects of administration of aqueous and methanolic extracts of leaves, stem and roots of *D.arborescens* and *C. dependens* on serum total protein of albino rats

Key: NC = Normal control, ST = Standard control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous.

4.1.8 *In vivo* antimalarial activities of the crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens*.

Results obtained from this test reveal that extracts of the leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* plants reduced parasite count in the mice infected with *Plasmodium berghei* (Table 4.18). Animals were inoculated on day zero (0), and the parasite count was determined on day 3 (72 hours after infection). Treatment commenced on the same day (day 3). From the results obtained, administration of the extracts significantly reduced ($p<0.05$) parasite count in all the treated groups by the 7th day (4 days after treatment) compared with the negative control (group 3) whose parasite count and percentage parasitemia significantly increased (Table 4.18 and 4.19). The experimental animals were further monitored till the 14th day (7 days after treatment); results obtained reveal that parasite count further reduced significantly ($p<0.05$) in the treated groups but increased in the negative control group. When compared with the standard antimalarial drug (artesunate), results obtained from the plant extracts differed significantly ($p<0.05$). Weakness was recorded in the negative control group from the 9th day; and some of the animals died before the 14th day (Table 4.20). Body weight of animals in all the treated groups increased. Among the groups treated with plants' extracts, the methanol extracts exhibited more activity than the aqueous extracts. Methanol extract of *Dictyandra arborescens* roots (received by animals in group 4) recorded the highest antimalarial activity by reducing parasite count from 63.0± 3.40 (75.2%) on the 3rd day to 33.0±1.30 (39.8%) on the 7th day, and subsequently 14.0± 0.85 (16.8%) on the 14th day and all the animals survived with increase in their body weight. The least antimalarial activity was recorded in those treated with aqueous extracts of the stem of the two plants (Table 4.18). *In vivo* antimalarial activities of the aqueous and methanol extracts of other parts of *Chasmanthera dependens* and *Dictyandra arborescens* plants are presented in Tables

4.18 and 4.19. The body weight of the animals treated with the extracts increased. Increase in the body weight was also recorded in animals in the normal and standard control groups. This was however not so for the infected but untreated animals (group 3) as their body weight decreased from 20 g to 17.5 g (Table 4.12).

Table 4.20: Effects of crude aqueous and methanolic extracts of the leaves, stem and roots, of *Chasmanthera dependens* and *Dictyandra arborescens* on parasite count of *Plasmodium berghi* infected male Swiss albino mice

Groups/Extracts	Parasite Count in Days			
	Before inoculation (Day 0)	Before Treatment (Day 3)	After Treatment (Day 7)	After Treatment (Day 14)
1. NC	0.00	$0^{b} \pm 0.00$	$0^{f} \pm 0.00$	$0^{f} \pm 0.00$
2. STD.C	0.00	$59^{a} \pm 2.00$	$4^{e} \pm 0.04$	$0^{f} \pm 0.00$
3. NEG.C	0.00	$59^{a} \pm 2.00$	$74^{a} \pm 2.39$	$99^{a} \pm 3.90$
4. DARM	0.00	$63^{a} \pm 3.40$	$33^{d} \pm 1.30$	$14^{e} \pm 0.85$
5. DALM	0.00	$61^{a} \pm 2.41$	$35^{d} \pm 1.33$	$18^{e} \pm 0.93$
6. DASM	0.00	$61^{a} \pm 2.48$	$42^{c} \pm 1.43$	$40^{b} \pm 1.41$
7. DARA	0.00	$60^{a} \pm 2.39$	$37^{c} \pm 1.35$	$25^{d} \pm 1.23$
8. DALA	0.00	$62^{a} \pm 2.45$	$38^{c} \pm 1.37$	$27^{d} \pm 1.26$
9. DASA	0.00	$62^{a} \pm 2.45$	$50^{b} \pm 1.52$	$42^{b} \pm 1.43$
10. CDRM	0.00	$63^{a} \pm$ 3..40	$32^{d} \pm 1.38$	$23^{d} \pm 1.21$
11. CDLM	0.00	$60^{a} \pm 2.39$	$35^{d} \pm 1.33$	$25^{d} \pm 1.23$
12. CDSM	0.00	$61^{a} \pm 2.48$	$48^{b} \pm 1.49$	$39^{b} \pm 1.39$
13. CDRA	0.00	$60^{a} \pm 2.39$	$36^{d} \pm 1.36$	$26^{d} \pm 1.25$
14. CDLA	0.00	$61^{a} \pm 2.48$	$42^{c} \pm 1.43$	$33^{c} \pm 1.30$
15. CDSA	00.00	$61^{a} \pm 2.48$	$51^{b} \pm 1.54$	$41^{b} \pm 1.42$

Values are average parasite count±SEM of 6 replicates. Mean values having different superscripts along the same column are significantly different ($P<0.05$).

Key: NC = Normal control, **STD.C** = Standard control, **NEG.C** = Negative control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous

Table 4.21: ***In vivo*** **antimalarial activities of crude aqueous and methanolic extracts of the roots, leaves and stems of *Chasmanthera dependens* and *Dictyandra arborescens* on percentage parasitemia of *Plasmodium berghei* infected male Swiss albino mice**

Groups/Extracts	**Average % Parasitemia**			
	Before inoculation (Day 0)	**Before Treatment (Day 3)**	**After Treatment (Day 7)**	**After Treatment (Day 14)**
1. NC	0.00	0.00	0.00	0.00
2. STD.C	0.00	69.0	4.6	0.00
3. NEG.C	0.00	63.0	72.4	79.2
4. DARM	0.00	75.2	39.8	16.8
5. DALM	0.00	72.8	41.4	21.2
6. DASM	0.00	73.2	51.4	48.4
7. DARA	0.00	72.0	43.4	31.0
8. DALA	0.00	74.6	45.2	32.0
9. DASA	0.00	77.4	60.0	49.8
10. CDRM	0.00	75.2	40.6	27.6
11. CDLM	0.00	68.2	40.2	30.2
12. CDSM	0.00	73.2	57.4	47.0
13. CDRA	0.00	67.0	42.8	31.2
14. CDLA	0.00	76.2	50.0	39.0
15. CDSA	00.00	76.4	61.4	49.4

Key: NC = Normal control, **STD.C** = Standard control, **NEG.C** = Negative control, **DARM** = D. *arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = D.arborescens leaves aqueous, **DASA** = D.arborescens stem aqueous, **CDRM** = C.dependens root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous

Table 4.22: Effects of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* on percentage body weight and survival rate of *Plasmodium berghi* infected male Swiss albino mice

Groups/ Extracts	Number of animals	Initial body Weight of animals (g)	Final body weight of animals (g)	% gain/loss in body weight of animals	% survival rate
1: NC	6	23.0	26.7	16.1	100
2: STD.C	6	18.0	20.8	15.6	100
3:NEG.C	6	20.0	17.5	12.5	33.3
4: DARM	6	21.0	23.6	12.4	100
5:DALM	6	19.0	21.2	11.6	100
6:DASM	6	21.0	22.8	8.6	66.7
7:DARA	6	19.0	21.0	10.5	100
8:DALA	6	20.0	22.0	11.0	100
9:DASA	6	23.0	24.5	6.5	50
10:CDRM	6	22.0	24.3	10.5	100
11:CDLM	6	22.0	24.1	9.6	100
12:CDSM	6	25.0	26.7	6.8	50
13:CDRA	6	24.0	26.4	10.0	100
14:CDLA	6	25.0	27.0	8.0	83.3
15:CDSA	6	24.0	25.4	5.8	50

Key: NC = Normal control, **STD.C** = Standard control, **NEG.C** = Negative control, **DARM** = *D. arborescens* root methanol, **DALM** = *D.arborescens* leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** = *D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, **CDRM** = *C.dependens* root methanol, **CDLM** = *C.dependens* leaves methanol, **CDSM** = *C.dependens* stem methanol, **CDRA** = *C.dependens* root aqueous, **CDLA** = *C. Dependens* leaves aqueous, **CDSA** = *C.dependens* stem aqueous

4.1.9 Result of Column chromatographic separation

Column chromatographic separation of methanolic extract of *D.arborescens* root yielded five (5) fractions (Table 4.23, Plate 4.1). These fractions had different colours - pale green (A), yellowish orange (B), pale brown (C), dark brown (D), and orange (E).

Table 4.23 Eluates obtained from methanol extract of *D.arborescens* roots using column chromatography at different solvent mixtures

Solvent Mixture	Colour of Eluates
H_{100}	Eluate A – Pale green
	Eluate B – Yellowish orange
H_{90}: C_{10}	-
H_{80}: C_{20}	-
H_{70}:C_{30}	-
H_{40}:C_{60}	-
H_{50}:C_{50} (1:1)	Eluate C - Pale brown
H_{40}: C_{60} (2:3)	Eluate D - Dark brown
H_{30}: C_{70}	-
H_{20}: C_{80}	-
H_{10}:C_{90}	-
C_{100}	-
C_{90}:M_{10}	-
C_{80}:M_{20} (4:2)	Eluate E – Orange

Key: H = hexane, C = Chloroform, M = methanol

E_AH_{100} = Eluate A, 100% hexane, E_BH_{100} = Eluate B, 100% hexane, E_CH_{50}:C_{50} = Eluate C, 50% Hexane**:** 50% Chloroform, E_DH_{40}**:**C_{60} = Eluate D, 40% Hexane**:** 60% Chloroform, E_EC_{80}**:**M_{20} = Eluate E, 80% Chloroform **:** 20% Methanol

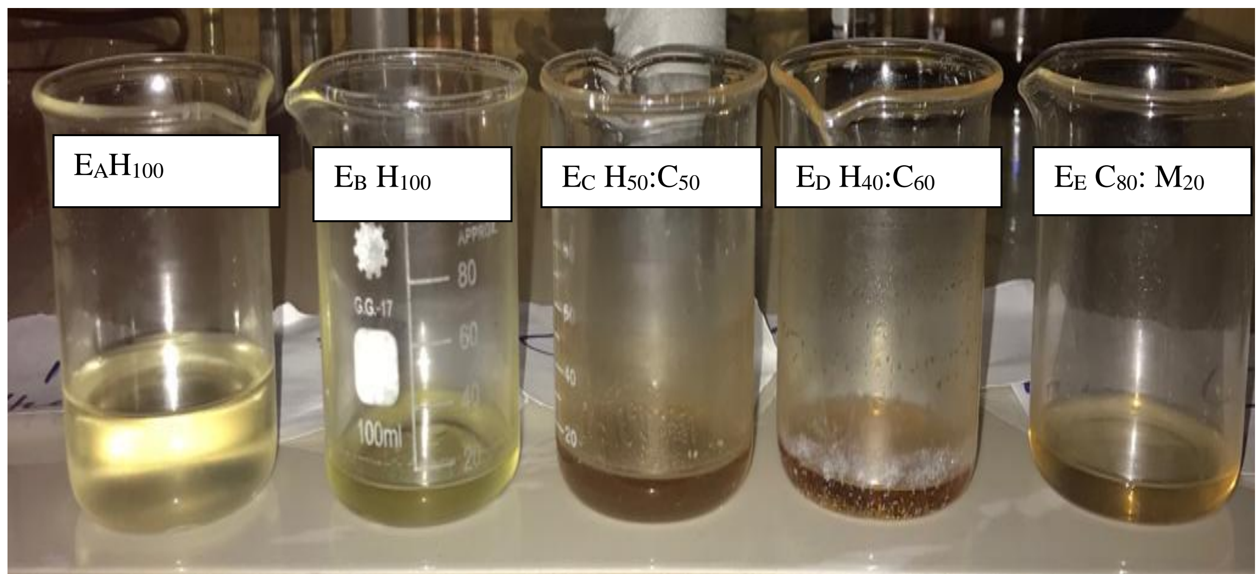

Plate 4.1: Colours/Codes of eluates obtained from methanolic extracts of *D.arborescens* root using column chromatography

Key: E_AH_{100} = Eluate A, 100% hexane, E_BH_{100} = Eluate B, 100% hexane, E_CH_{50}:C_{50} = Eluate C, 50% Hexane**:**50% Chloroform, E_DH_{40}**:**C_{60}=Eluate D, 40% Hexane**:**60% Chloroform, E_EC_{80}**:**M_{20} = Eluate E, 80% Chloroform **:** 20% Methanol

4.1.10 Results of antimalarial activities of eluates obtained from column chromatography

Results obtained in this test reveal that all eluates reduced parasite count in mice infected with *Plasmodium berghei* (Table 4.24). Animals were inoculated on day zero (0), and the parasite count was determined on day 3 (72 hours after infection). Treatment commenced on the same day (day 3). Results obtained show that administration of the eluates significantly reduced ($p<0.05$) parasite count in all the treated groups by the 7th day (4 days after treatment), compared to the negative control (group 3). Eluate A coded 'E_AH_{100}' showed the highest antimalarial activity by reducing percentage parasitemia from 50% on day 3 to 30.4% on day 7 and 14.8% on day 14 (Table 4.24). Activity of this eluate differed significantly ($p<0.05$) from that of the other eluates.

However, when compared with the standard antimalarial drug (artesunate), antimalarial activity of this eluate was lower.

Table 4.24: Antimalarial activities of eluates/fractions obtained from column chromatography of methanol extract of *D.arborescens* roots

Groups/ Eluates	Parasite Count in Days			
	Before inoculation (Day 0)	**Before Treatment (Day 3)**	**After Treatment (Day 7)**	**After Treatment (Day 14)**
1. NC	0.00	$0^{b}\pm 0.00$	$0^{h}\pm 0.00$	$0^{g}\pm 0.00$
2. STD.C	0.00	$86.67^{a}\pm0.14$	$8.67^{g}\pm0.18$	$0^{g}\pm0.00$
3. NEG.C	0.00	$83.67^{a}\pm0.31$	$103.33^{a}\pm0.37$	$128.0^{a}\pm0.50$
4. Eluate A (E_AH_{100})	0.00	$83.33^{a}\pm0.31$	$50.67^{f}\pm0.21$	$24.67^{f}\pm0.32$
5.Eluate B (E_BH_{100})	0.00	$84.0^{a}\pm0.17$	$67.0^{d}\pm0.58$	$55.33^{d}\pm0.27$
6. Eluate C ($E_CH_{50}:C_{50}$)	0.00	$82.50^{a}\pm2.00$	$62.67^{e}\pm0.16$	$47.60^{e}\pm0.21$
7. Eluate D ($E_DH_{40}:C_{60}$)	0.00	$84.33^{a}\pm0.39$	$71.67^{c}\pm0.02$	$60.67^{c}\pm0.27$
8. Eluate E ($E_EC_{80}:M_{20}$)	0.00	$87.0^{a}\pm0.14$	$76.67^{b}\pm0.18$	$68.67^{b}\pm0.19$

Values are average parasite count±SEM of 6 replicates. Mean values having different superscripts along the same column are significantly different (P<0.05).

Key: NC = Normal control, STD.C = Standard control, E_AH_{100} = Eluate A, 100% hexane, E_BH_{100} = Eluate B, 100% hexane, $E_CH_{50}:C_{50}$ = Eluate C, 50%Hexane:50% Chloroform, $E_DH_{40}:C_{60}$=Eluate D, 40% Hexane:60% Chloroform, $E_EC_{80}:M_{20}$ = Eluate E, 80% Chloroform : 20%Methanol

Table 4.25: Effects of the various eluates/fractions on percentage parasitemia

Groups/Eluates	Average % Parasitemia			
	Before inoculation (Day 0)	**Before Treatment (Day 3)**	**After Treatment (Day 7)**	**After Treatment (Day 14)**
1. NC	0.00	0.00	0.00	0.00
2. STD.C	0.00	52.0	5.2	0.00
3. NEG.C	0.00	50.2	62.0	76.8
4. EluateA (E_AH_{100})	0.00	50	30.4	14.8
5. Eluate B (E_BH_{100})	0.00	50.4	40.2	33.2
6.Eluate C (E_CH_{50}:C_{50})	0.00	49.5	37.6	28.6
7.Eluate D (E_DH_{40}:C_{60})	0.00	50.6	43	36.4
8.Eluate E (E_EC_{80}:M_{20})	0.00	52.2	46	41.2

Key: NC = Normal control, STD.C = Standard control, E_AH_{100} = Eluate A, 100% hexane, E_BH_{100} = Eluate B, 100% hexane, E_CH_{50}:C_{50} = Eluate C, 50% Hexane**:** 50% Chloroform, E_DH_{40}**:**C_{60} = Eluate D, 40% Hexane**:**60% Chloroform, E_EC_{80}**:**M_{20} = Eluate E, 80% Chloroform **:** 20% Methanol

4.1.11 GC-FID analysis of hexane fractions A and B from methanolic extracts of *D.arborescens* roots

Eluate A (E_AH_{100}) obtained from hexane soluble fraction of methanol extract of *D.arborescens* roots recorded the highest antimalarial activity. This eluate was sub-fractionated using column chromatography to obtain purer eluates. This process yielded two eluates coded eluate A (**E_A)** and eluate B (**E_B)**. GC-FID analysis of eluate A (**E_A**) revealed presence of proanthocyanin, naringin, cohumulone, anthocyanin, spartein, sapogenin, flavonones, steroids, kaempferol, flavones, cathechin, and tannin (Fig. 4.22 and Table 4.26). The highest quantities of phytochemicals for eluate A (**E_A**) were 16.62%, 15.16% and 11.34% recorded for kaempferol, anthocyanin and proanthocyanin respectively (Table 4.26). The second eluate B (**E_B**) recorded presence of the following phytochemicals- proanthocyanin, naringin, epihedrine, anthocyanin, humulone, spartein, sapogenin, steroids, epicatechin, kaempferol, flavones, catechin and resveratol (Fig. 4.23 and Table 4.27). Phytochemicals found in high quantities include Anthocyanin (28.71%), kaempferol (25.57%), and flavones (12.44%) (Table 4.25).

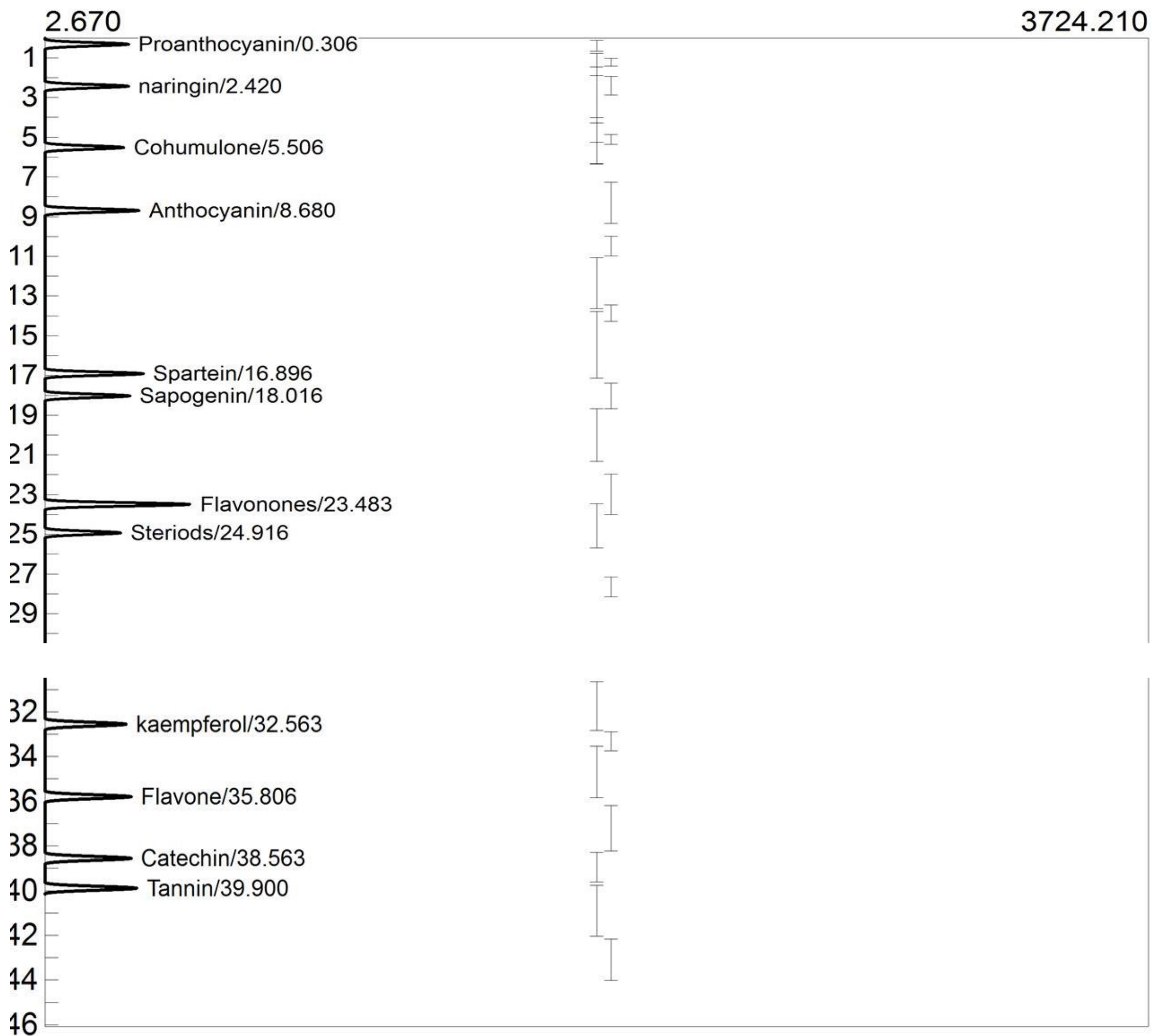

Figure 4.22: Chromatogram showing the phytochemical constituents of eluate A (**E$_A$**) from hexane fraction of methanolic extracts of *D.arborescens* roots

Table 4.26: Phytochemical components identified in eluate A (E_A) by GC-FID

Phytoconstituents	PK	RT	Area	Height	Conc (µg/ml)	% Composition
Proanthocyanin	1	0.306	3658.8756	285.288	16.1987	11.34
Naringin	2	2.420	3648.9914	285.407	15.6082	10.92
Cohumulone	3	5.506	3437.7298	269.451	11.1854	7.82
Anthocyanin	4	8.680	4088.1871	320.688	21.6556	15.16
Spartein	5	16.896	4255.4497	334.689	3.4184	2.39
Sapogenin	6	18.016	3691.6775	289.355	2.4686	1.73
Flavonones	7	23.483	6295.9118	493.015	9.6244	6.74
Steroids	8	24.916	3293.2822	258.449	3.4053	2.38
Kaempferol	9	32.563	3529.8758	277.175	28.0363	16.62
Flavone	10	35.806	3738.7696	293.650	9.4031	6.58
Catechin	11	38.563	3750.8060	294.034	10.4116	7.29
Tannin	12	39.900	3964.3128	312.169	11.4660	8.02
					142.8817	

Key: PK =Peak, RT =Retention time

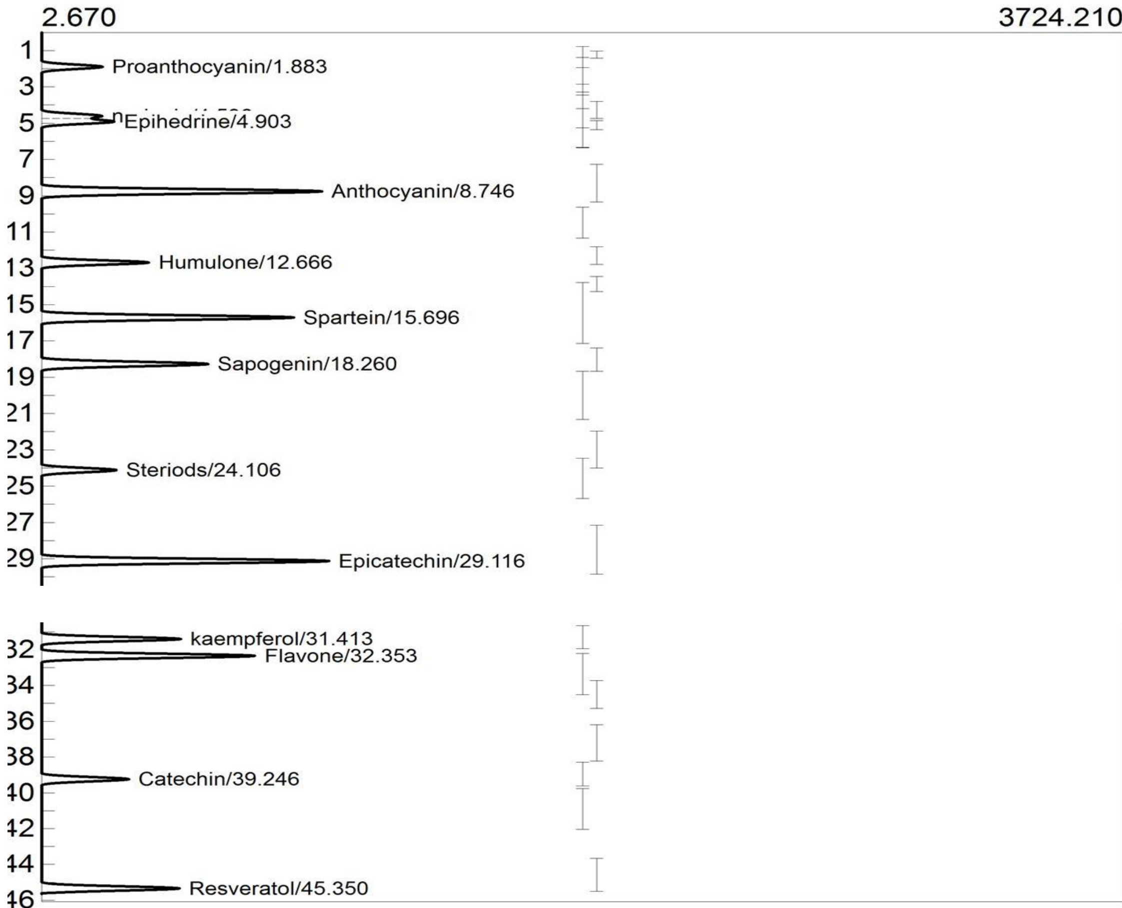

Figure 4.23: Chromatogram showing the phytochemical constituents of eluate B (**E_B**) from hexane fraction of methanolic extracts of *D.arborescens* roots

Table 4.27: Phytochemical components identified in eluate B (E_B) by GC-FID

Phytoconstituents	PK	RT	Area	Height	Conc (µg/ml)	% Composition
Proanthocyanin	1	1.883	3733.4476	212.018	16.5289	6.24
Naringin	2	4.596	3462.5282	210.938	14.8107	5.59
Epihedrin	3	4.903	4523.6006	253.295	3.5952	1.36
Anthocyanin	4	8.746	17782.1411	968.052	76.0076	28.71
Humulone	5	12.666	6589.1400	373.983	5.4272	2.05
Spartein	6	15.696	15646.2445	872.729	12.5686	4.75
Sapogenin	7	18.260	10783.8175	578.227	7.2109	2.72
Steroids	8	24.106	4628.3302	262.965	4.7858	1.81
Epicatechin	9	29.116	17749.1294	994.076	0.0000	0.00
Kaempferol	10	31.413	8523.0996	483.918	67.6955	25.57
Flavone	11	32.353	13097.7557	739.537	32.9411	12.44
Catechin	12	39.246	5361.1156	304.460	14.8816	5.62
Resveratol	13	45.350	8269.4048	477.570	8.3223	3.14
					264.7753	

Key: PK = Peak, RT = Retention time

4.1.12 FTIR Analysis of eluates E_A and E_B from hexane fraction of methanolic extracts of *D.arborescens* roots

Fourier transform Infrared spectrophotometer (FTIR) works on the principle that bonds between different elements absorb light at different frequencies. The light is measured using an infrared spectrometer which produces output of an infrared spectrum. It is one of the important tools in identifying the type of functional groups present in unknown compounds. The spectra wavelength obtained in the FTIR analysis of hexane fraction of *D.arborescens* roots characterized the inherent functional groups present in the 2 eluates (Fig 4.24 and 4.25). The Infra-red spectra of eluates $\mathbf{E_A}$ and $\mathbf{E_B}$ showed different peaks, signifying transitions between vibration levels of different molecules. A total of 5 major peaks were recorded in eluate A ($\mathbf{E_A}$) while 6 major peaks were recorded in eluate B ($\mathbf{E_B}$). These peaks were assigned to different functional groups based on their values (Table 4.28 and 4.29).

Table 4.28: FTIR peak values and functional groups obtained from eluate A (E_A) of hexane fraction of methanolic extract of *Dictyandra arborescens* roots.

S/N	Peak value/wavelength (cm^{-1})	Functional group	Type of bond
1.	2922.2	Alkanes ($-CH_2-$)	C-H Stretch
2.	2855.1	Alkanes ($-CH_2-$)	C-H stretch
3.	1736.9	Esters	C=O Stretch
4.	1461.1	Alkanes ($-CH_3$)	C-H def.
5.	1379.1	Alkanes (CH_3)	C-H def.
6.	1243.7	Aliphatic esters (CH_3COOR)	-
7.	1185.3	Esters (H-COOR)	-

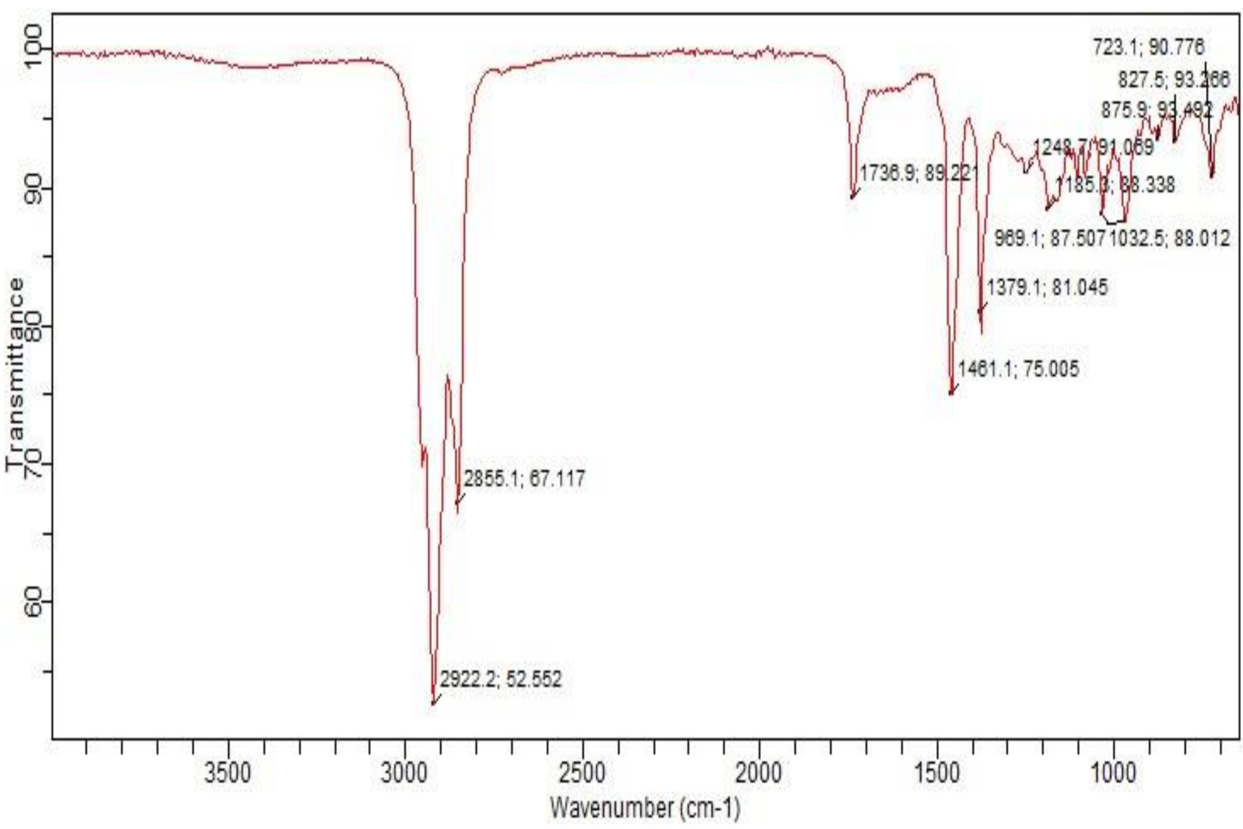

Figure 4.24: FTIR Spectrum of eluate A (E_A) from hexane fraction of methanolic extract of *Dictyandra arborescens* roots.

Table 4.29: FTIR peak values and functional groups obtained from eluate B (E_B) of hexane fraction of methanolic extract of *Dictyandra arborescens* roots.

S/N	Peak value/wavelength (cm^{-1})	Functional group	Type of bond
1.	3008.0	Esters	OC-H stretch
2.	2922.2	Alkanes ($-CH_2-$)	C-H stretch
3.	2855	Alkanes ($-CH_2-$)	C-H stretch
4.	1718.3	α- Halogeno carboxylic acids	C=O stretch
5.	1625.1	Non-conjugated alkenes	C=C stretch
6.	1461.1	Alkanes ($-CH_3$)	C-H def.

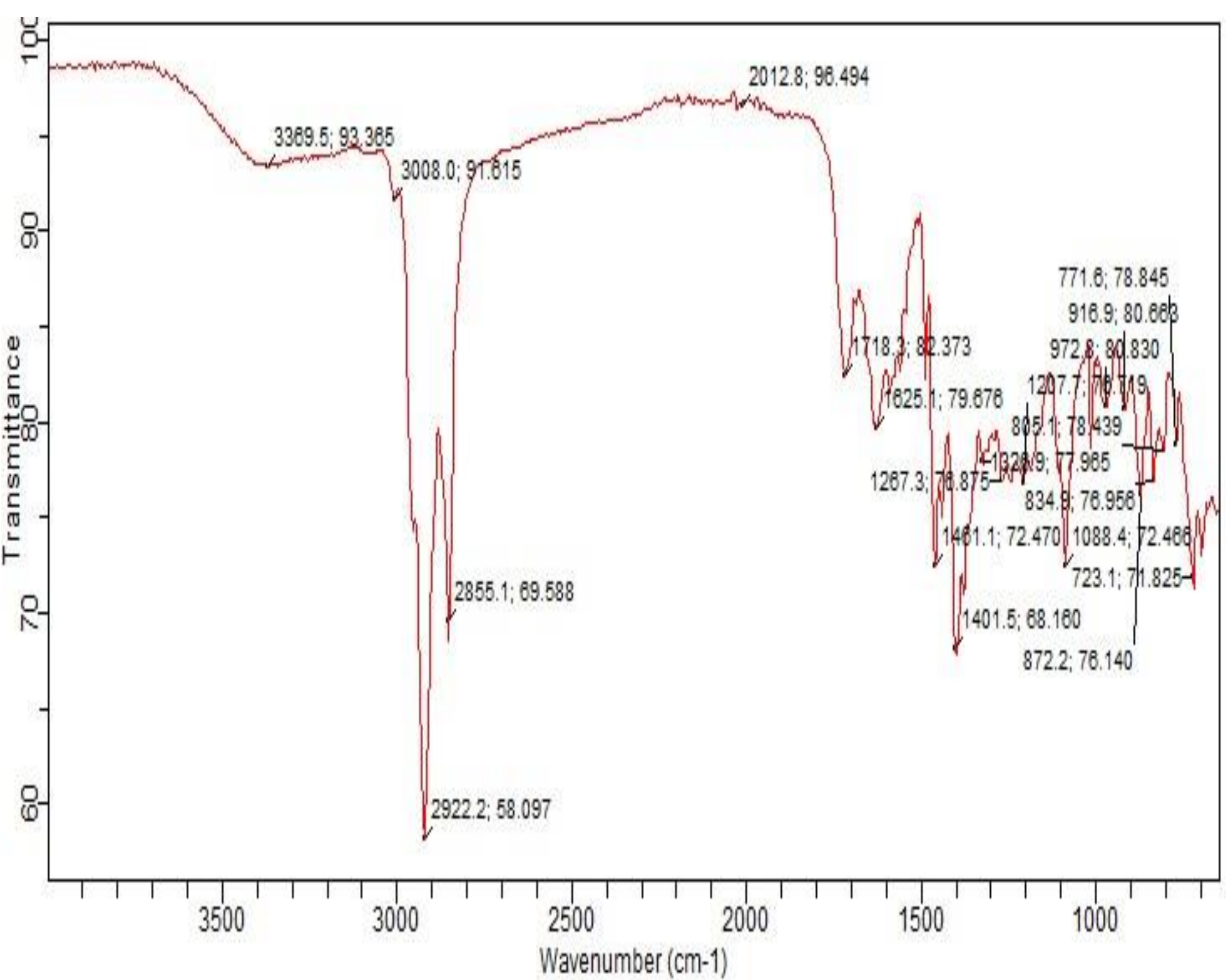

Figure 4.25: FTIR Spectrum of eluate B (E_B) from hexane fraction of methanolic extract of *Dictyandra arborescens* roots.

4.1.13 GC-MS Analysis of eluate A (E_A) and eluate B (E_B) of hexane fraction of methanol extract of *D.arborescens* roots

Some of the compounds obtained in eluate A (**E_A**) include propane 1,2 dichloro propane, hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,17 octadecadienal, (Z)-, cis-13-octadecenoic acid, methyl ester, 6-octadecenoic acid, methyl ester, (Z)-, heptadecanoic acid, 16-methyl, methyl ester and Bis (2-ethylhexyl) phtalate whose mass spectrums are presented in Figures 4.27 to 4.37. Compounds obtained from eluate B (**E_B**) include carbonic acid, prop-1-en-2-yl tetradecyl ester, 5-octadecene, (E)-, isobutyl tetradecyl carbonate, hexadecanoic acid, methyl ester, n-hexadecanoic acid butyl octadecyl ether, 10-octadecenoic acid, methyl ester, cyclopropaneoctanoic acid, 2-hexyl-2,3-divinyloxirane and carbonic acid, dodecyl 2,2,2-trichloroethyl ester whose mass spectrums are presented in Figures 4.39 to 4.53. The most abundant compounds in eluate A were 6-Octadecenoic acid methyl ester and Hexadecanoic acid methyl ester whose percentage constituents were 36.80% and 28.20% respectively. For eluate B, the most abundant compounds were 10-Octadecenoic acid, methyl ester and n-Hexadecanoic acid with percentage constituents of 20.70% and 15.29%. Hexadecanoic acid, methyl ester and n-hexadecanoic acid were present in both eluates (Tables 4.30 and 4.31).

These bioactive compounds exhibit varying pharcological actions on biological systems, such as, antimicrobial, antimalarial, antioxidant, anti-inflammation, anticancer, hepato protective activities etc

Table 4.30: Molecular formulae, weight and structures of compounds identified in GC-MS analysis of eluate A (E_A) from hexane fraction of methanol extract of *D.arborescens* roots

PK	RT	Name of compound	Molecular structure	Molecular Formular	Molecular Weight (g/mol)	Percentage (%)
1	7.10	1,2-dichloropropane		$C_3H_6C_{12}$	112.98	1.14
2	8.04	Cis-alpha-bisabolene		$C_{12}H_{24}$	204.35	1.32
3	9.02	Dichloroacetic acid tridecyl ester		$C_{15}H_{28}Cl_2O_2$	311.3	0.91
4	11.40	1-Octadecene		$C_{18}H_{36}$	252.5	0.61
5	12.42	Hexadecanoic acid methyl ester		$C_{17}H_{34}O_2$	270.45	28.20
6	13.53	n-hexadecanoic acid		$C_{16}H_{32}O_2$	256.43	2.30
7	15.00	9,17-Octadecadienal, (Z)-		$C_{19}H_{34}O_2$	294.47	3.55
8	15.08	Cis-13-Octadecenoic acid		$C_{21}H_{40}O_2$	324.5	15.77
9	16.17	6-Octadecenoic acid methyl ester, (Z)-		$C_{19}H_{36}O_2$	282.47	36.80
10	15.59	Heptadecanoic acid, 16 methyl-, methyl ester		$C_{19}H_{38}O_2$	298.5	3.61
11	21.88	Bis(2-ethylhexyl) phtalate		$C_{24}H_{38}O_4$	390.56	5.79

Key: PK = Peak, RT = Retention time

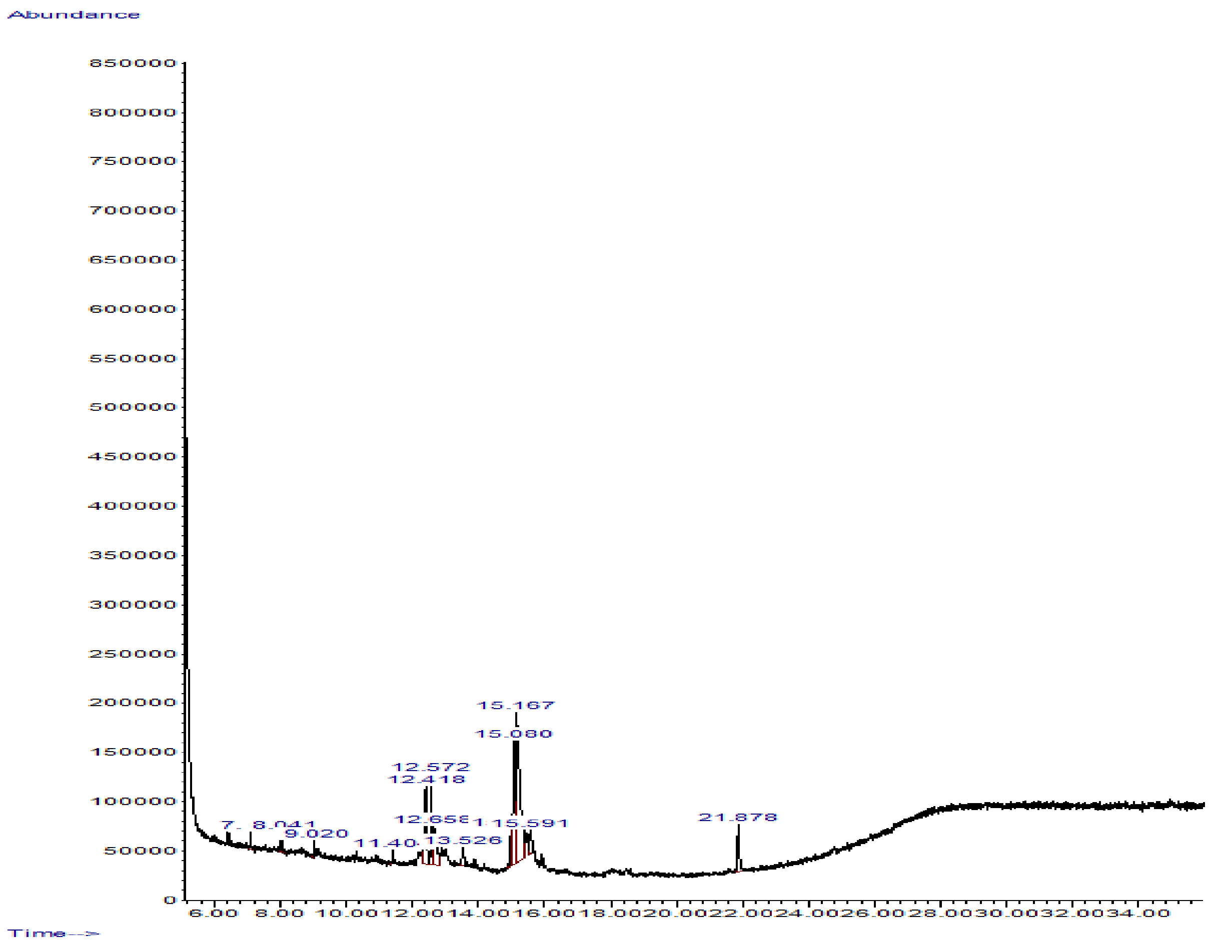

Figure 4.26: GC-MS profile of eluate A (E_A) from hexane fraction of methanol extract of *D.arborescens* roots

Mass spectrum of various compounds identified by GC-MS analysis of eluate A **(E_A)** are presented below:

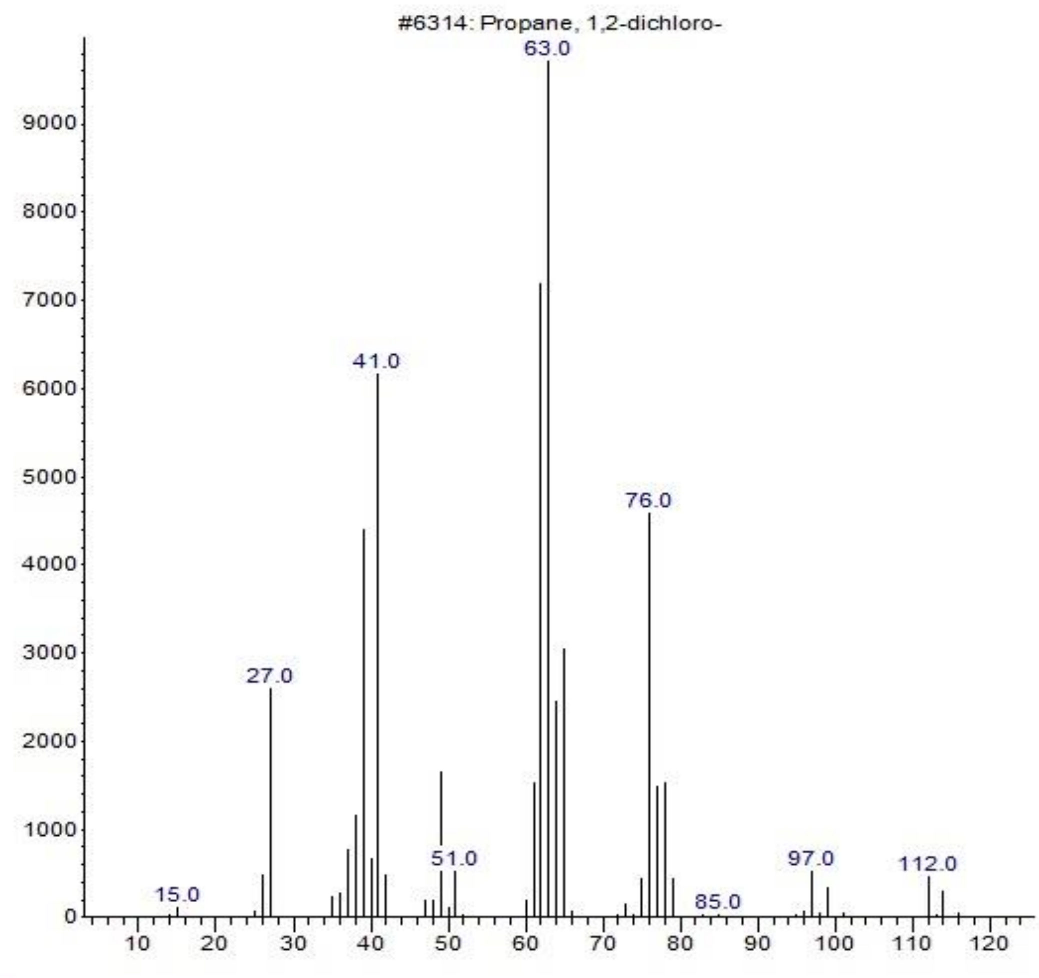

Figure 4.27: Mass spectrum of 1,2 -dichloropropane

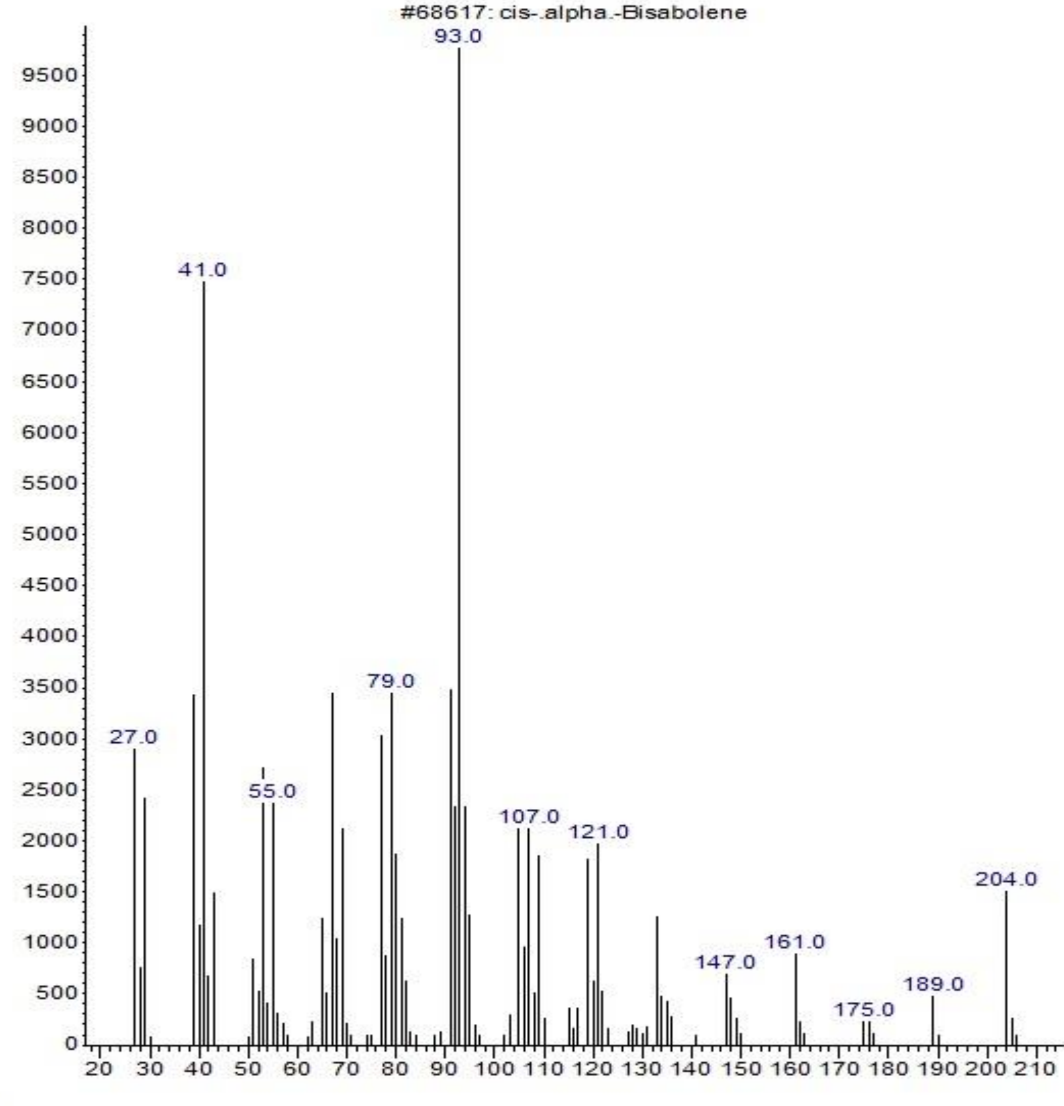

Figure 4.28: Mass spectrum of cis-alpha-Bisabolene

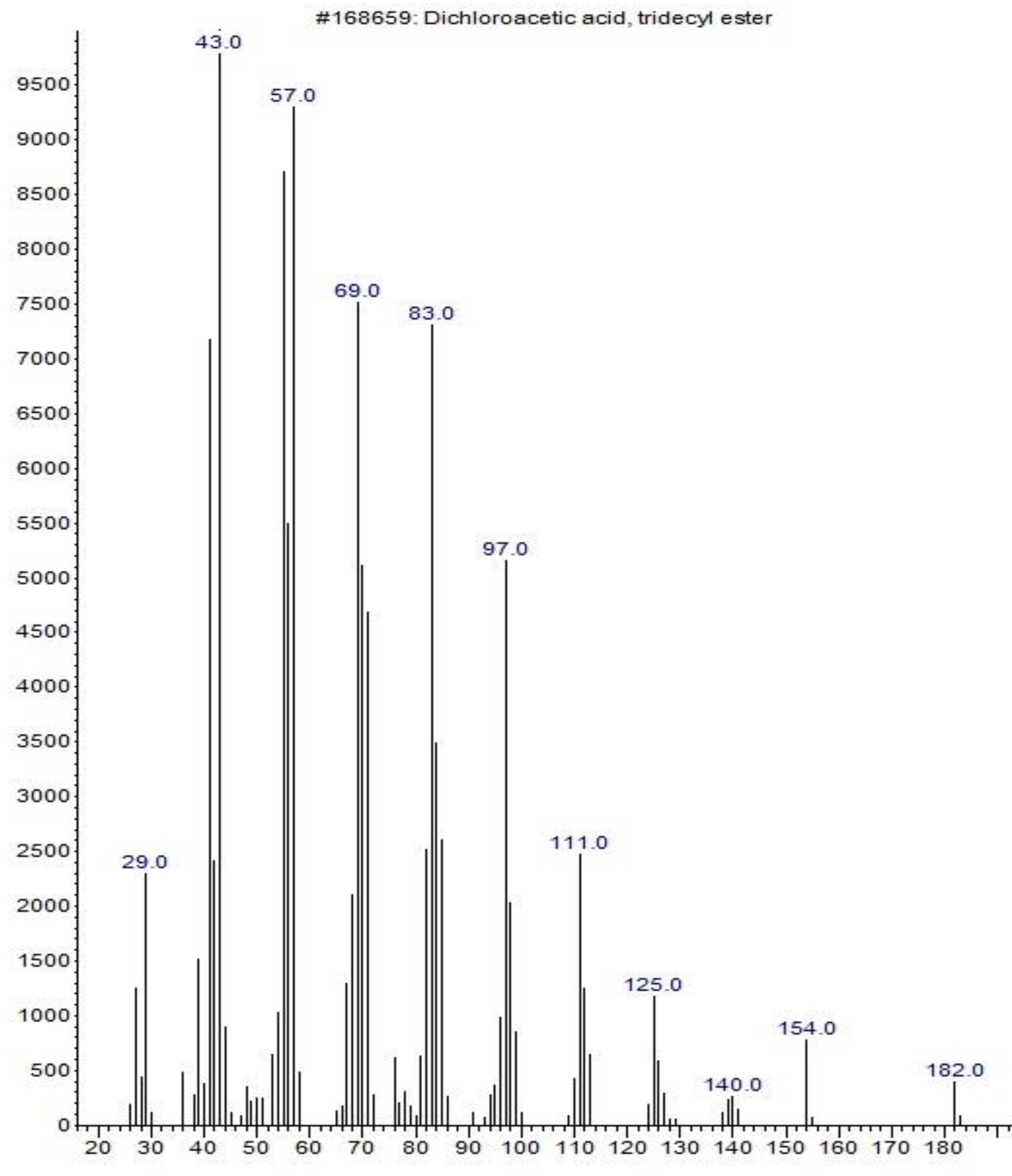

Figure 4.29: Mass spectrum of Dichloro acetic acid, tridecyl ester

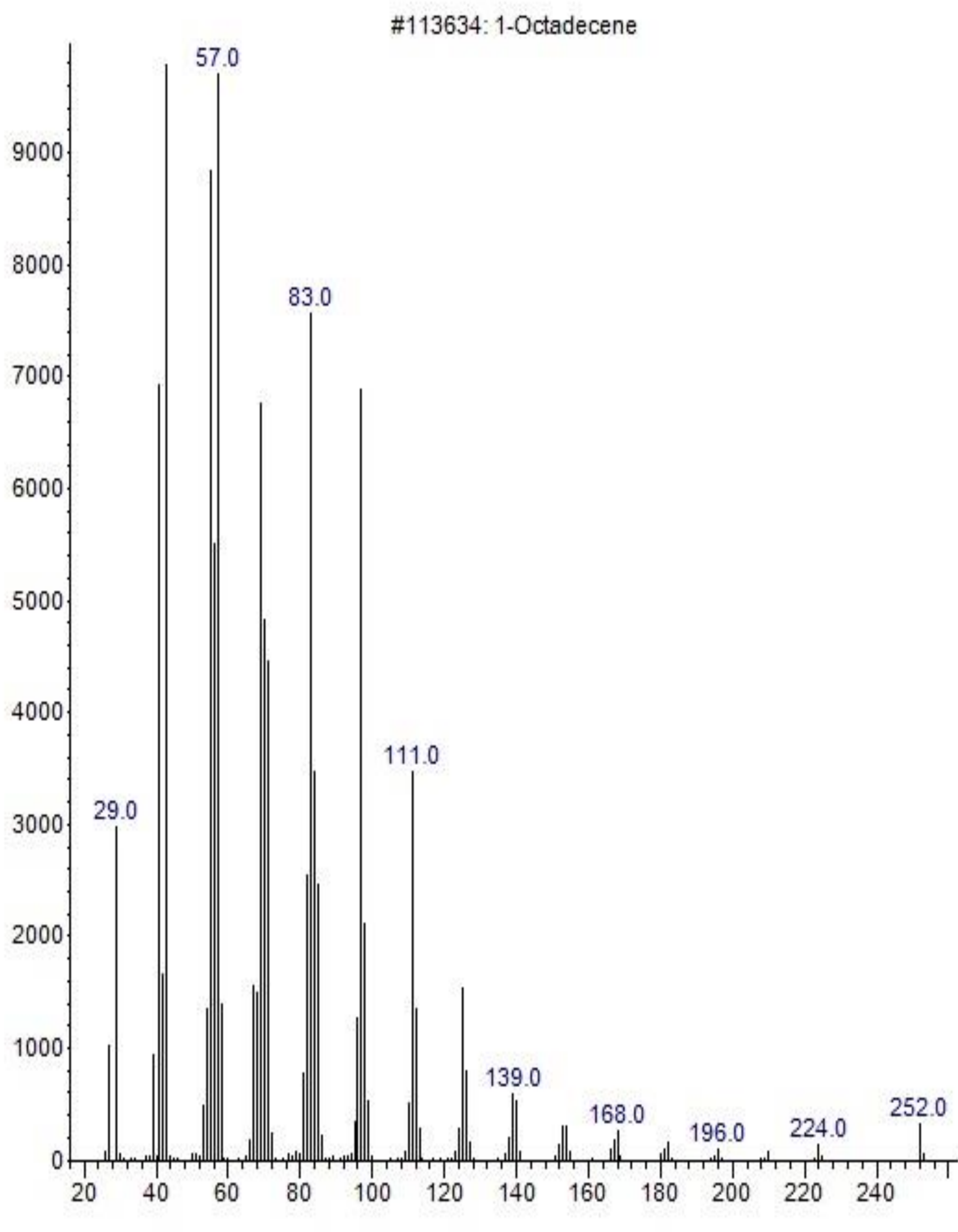

Figure 4.30: Mass spectrum of 1-Octadecen

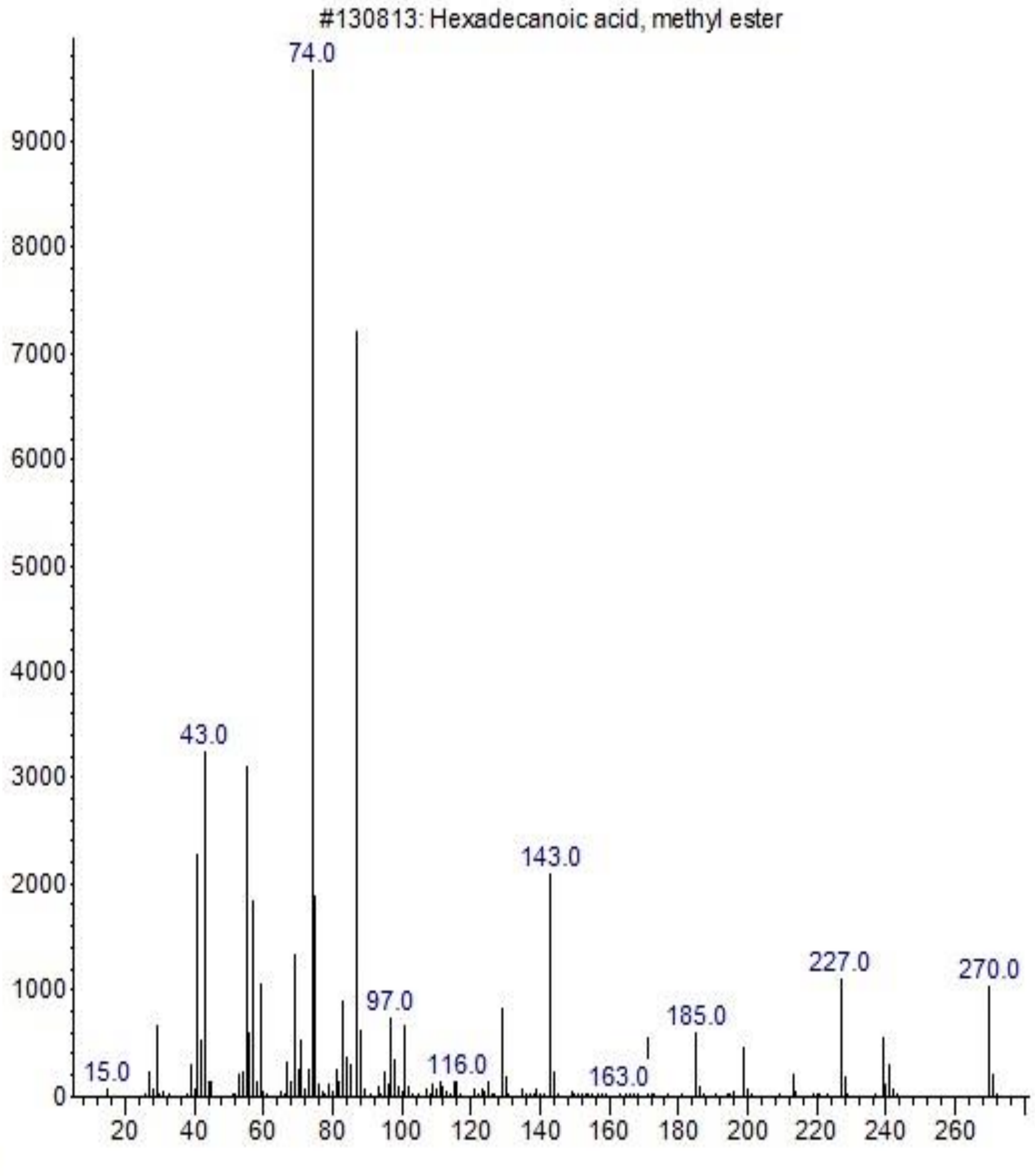

Figure 4.31: Mass spectrum of Hexadecanoic acid, methyl ester

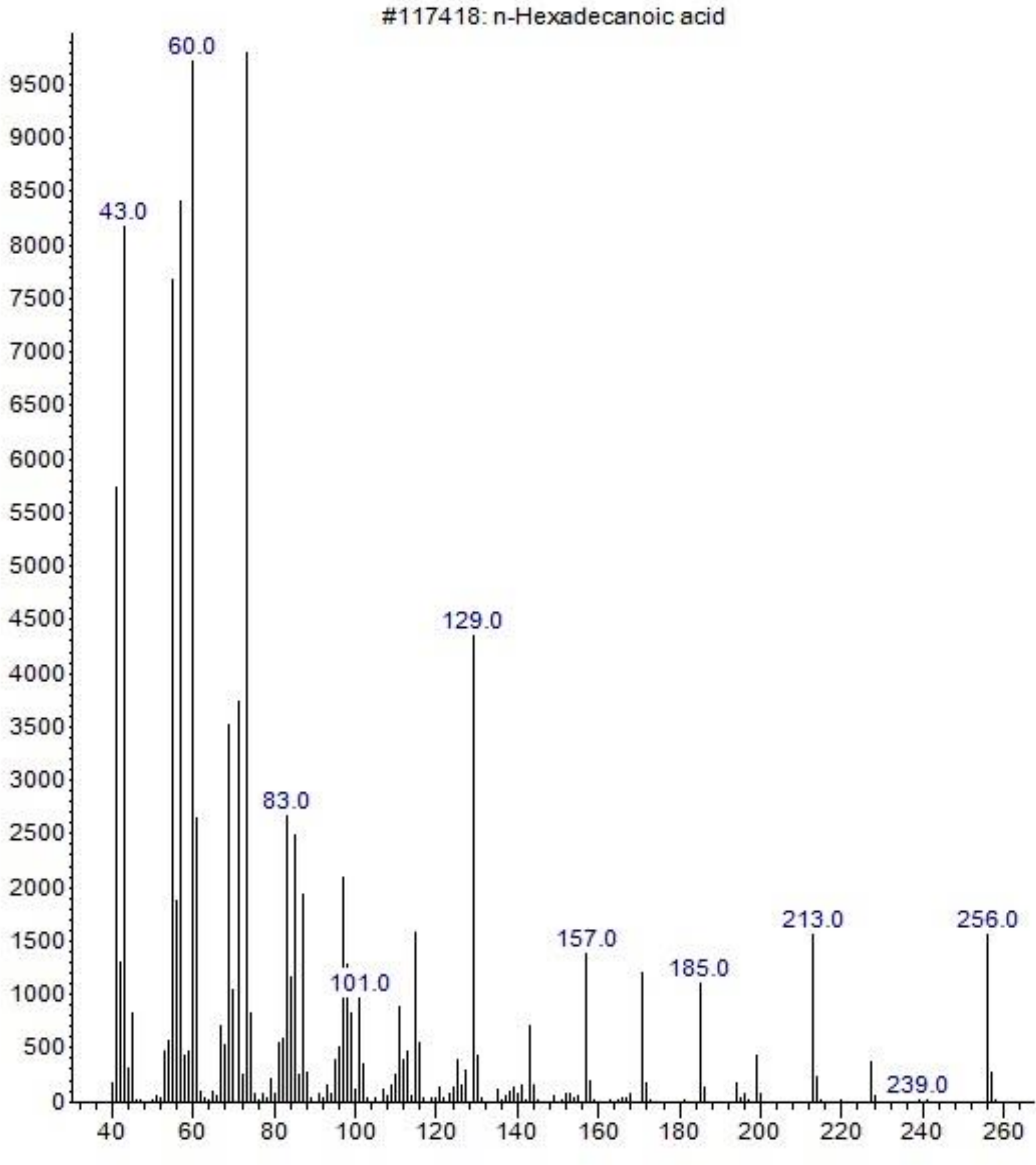

Figure 4.32: Mass spectrum of n-Hexadecanoic acid

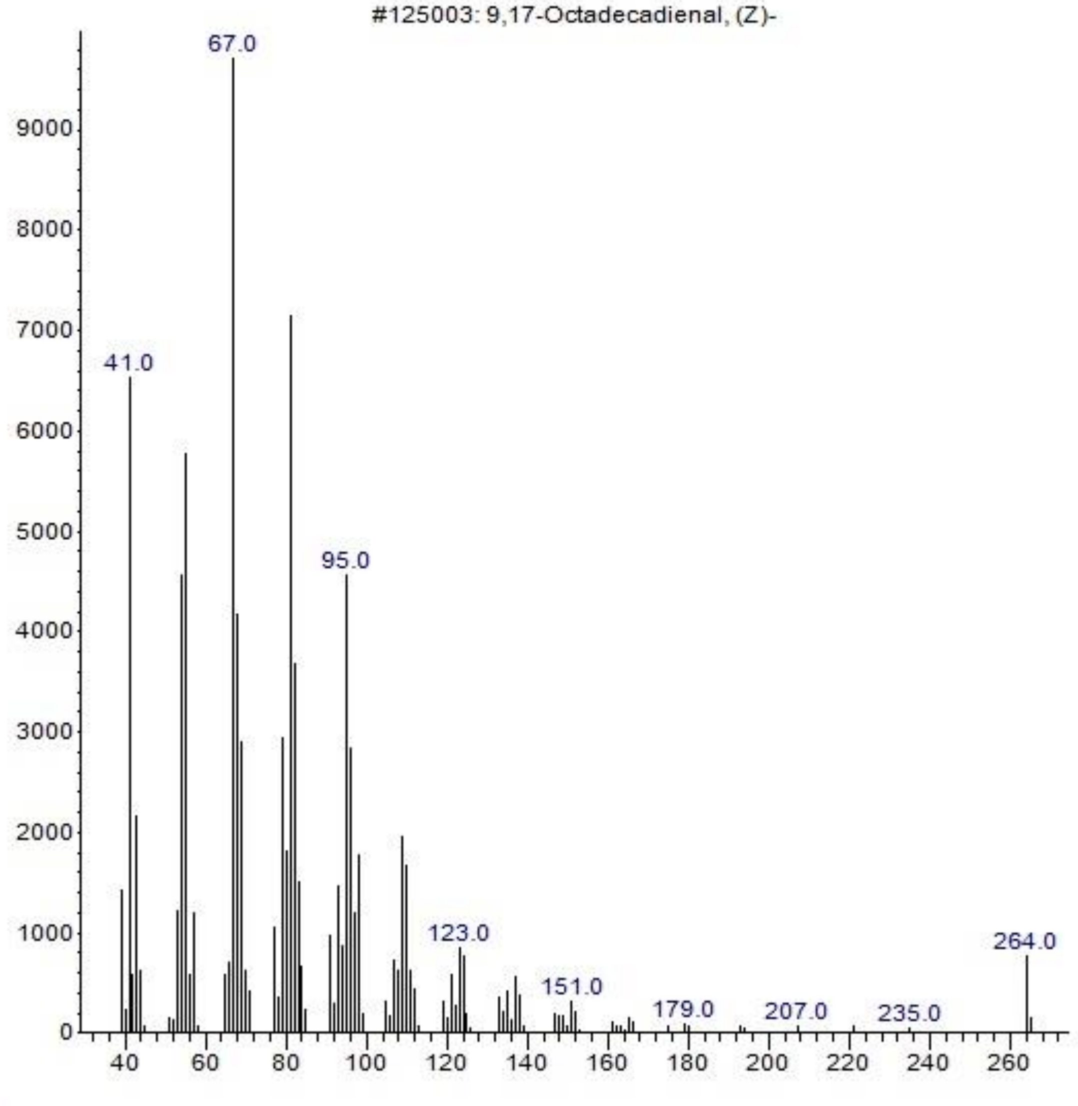

Figure 4.33: Mass spectrum of 9,17-Octadecadienal, (Z)-

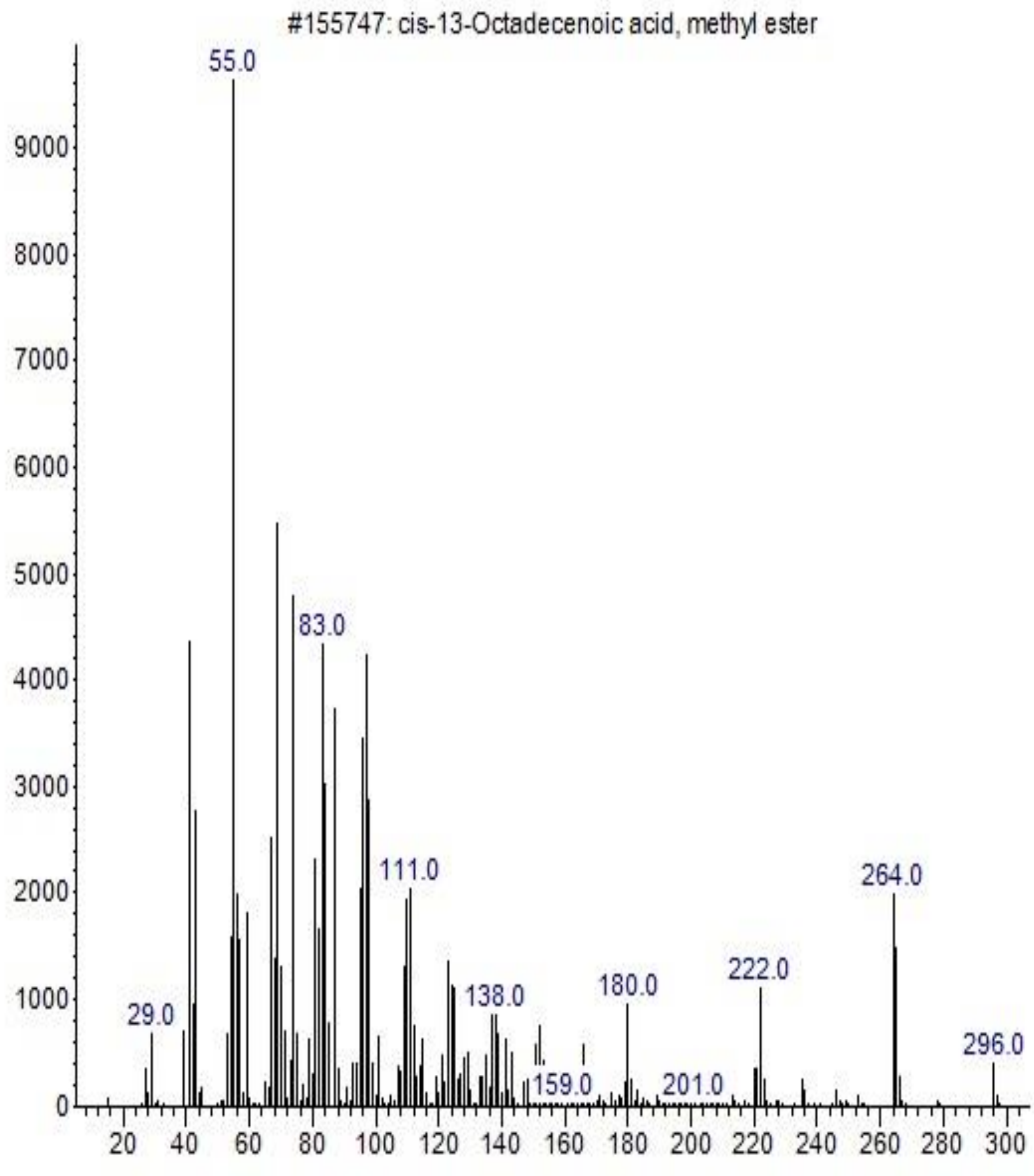

Figure 4.34: Mass spectrum of cis-13-Octadecenoic acid, methyl ester

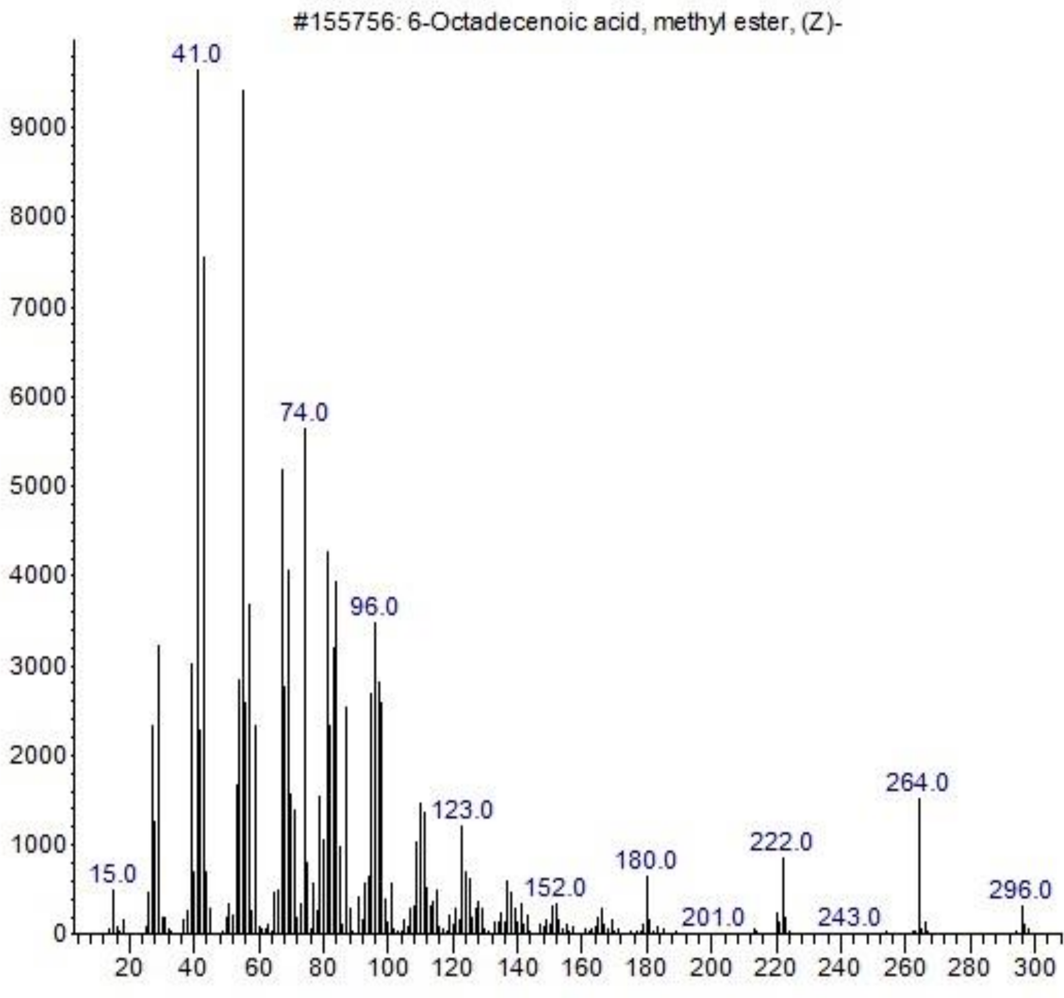

Figure 4.35: Mass spectrum of 6-Octadecenoic acid, methyl ester, (Z)-

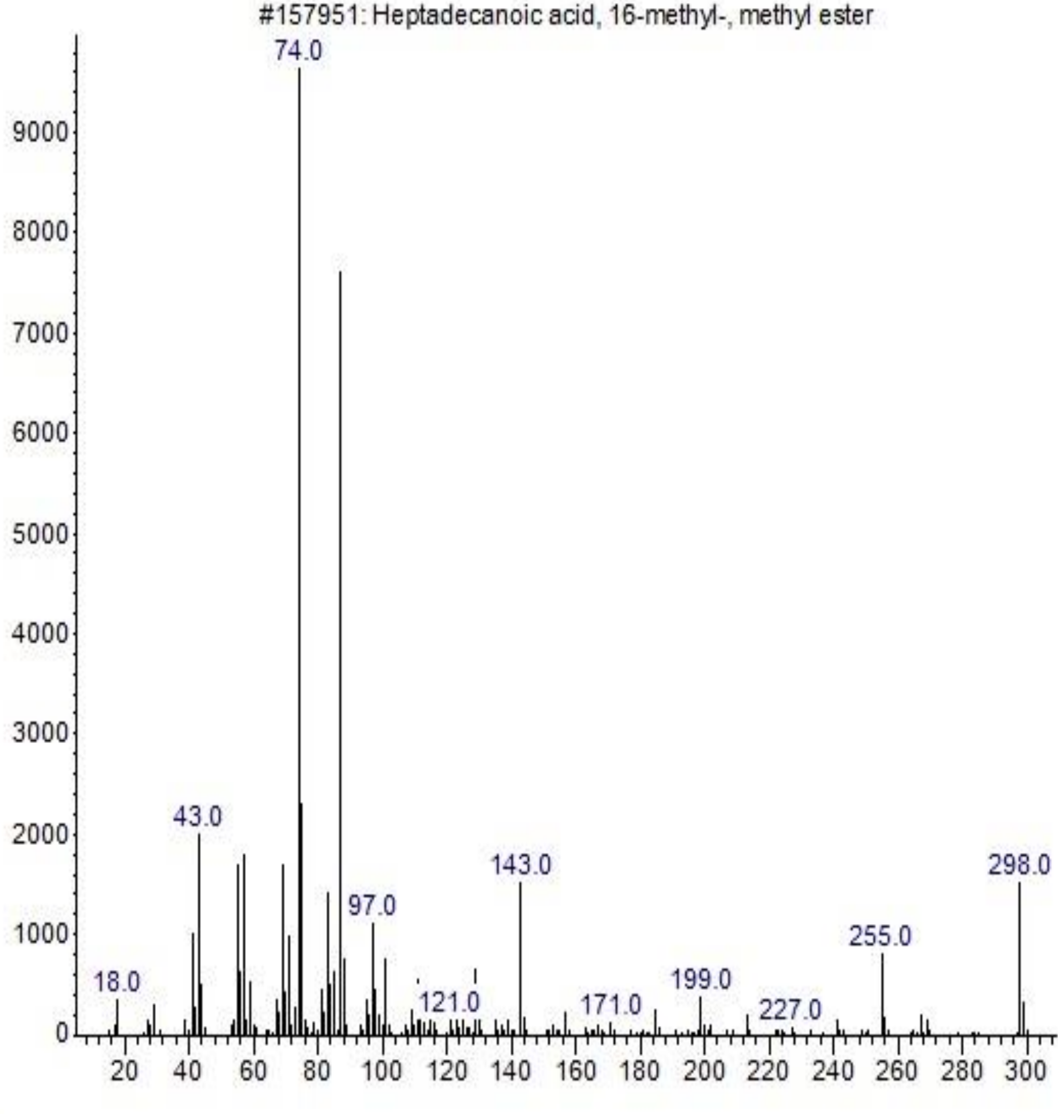

Figure 4.36: Mass spectrum of Heptadecanoic acid, 16 methy-, methyl ester

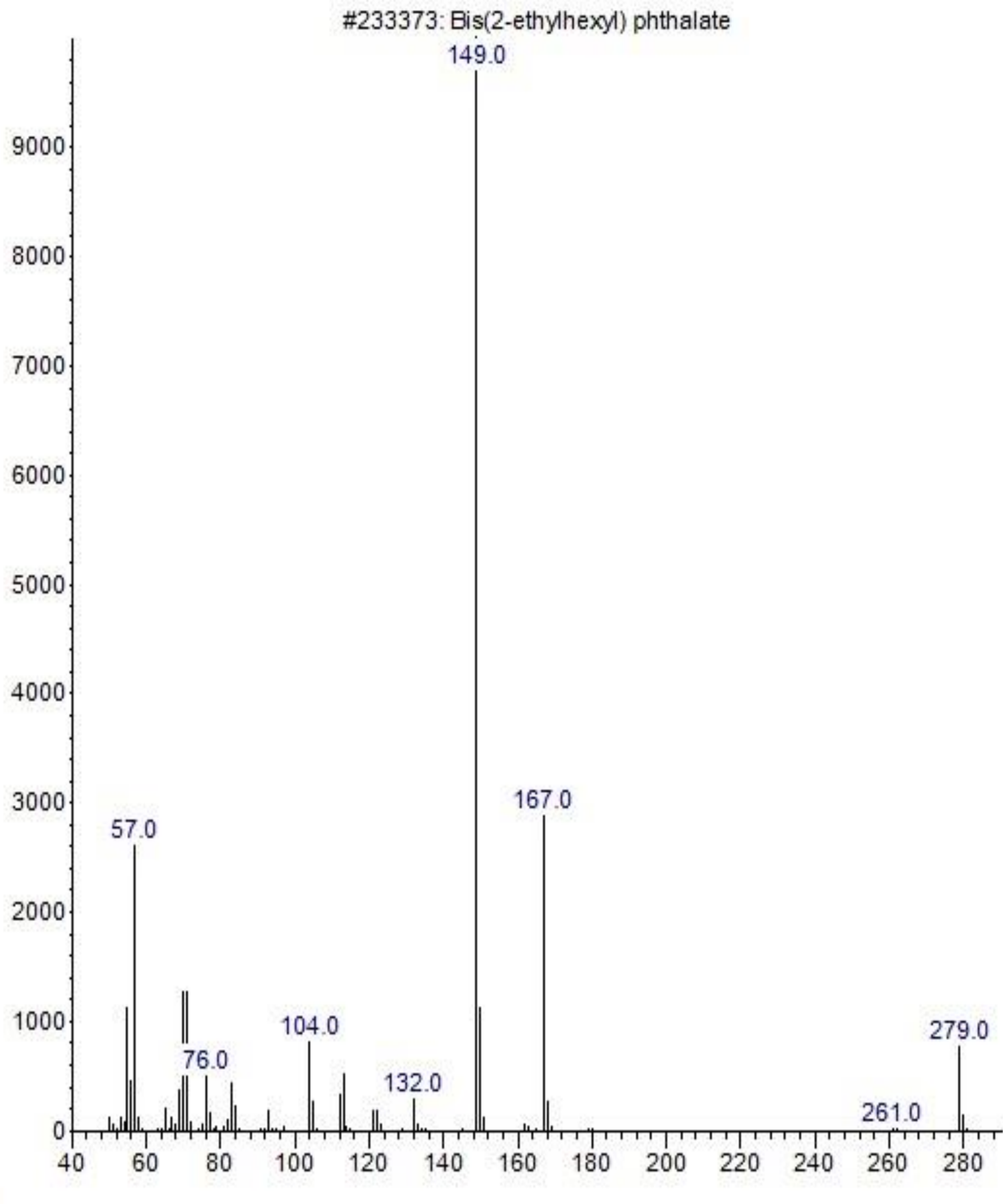

Figure 4.37: Mass spectrum of Bis(2-ethylhexyl) phtalate

Table 4.31: Compounds identified in GC-MS analysis of eluate B (E_B) from hexane fraction of methanol extract of *D.arborescens* roots

PK	RT	Name of compound	Molecular structure	Molecular Formular	Molecular Weight (g/mol)	Percentage (%)
1.	6.4154	Carbonic acid, prop-1-en-2-yl tetradecyl ester		$C_{17}H_{32}O_3$	284.43	4.55
2.	9.0105	5-Octadecene, (E)-		$C_{18}H_{38}$	252.5	4.67
3.	11.3955	Isobutyl tetradecyl carbonate		$C_{19}H_{38}O_3$	314.5	3.90
4.	13.0093	Hexadecanoic acid, methyl ester		$C_{17}H_{34}O_2$	270.45	10.47
5.	13.5262	n-Hexadecanoic acid		$C_{16}H_{32}O_2$	256.43	15.29
6.	13.8711	Butyl octadecyl ether		$C_{22}H_{46}O$	326.60	2.90
7.	15.3452	10-Octadecenoic acid, methyl ester		$C_{19}H_{36}O_2$	296.48	20.70
8.	15.4072	Cyclopropaneoctanoic acid, 2-hexyl-,methyl ester		$C_{20}H_{38}O_2$	310.5	3.12
9.	15.6873	Methyl tetradecanoate		$C_{15}H_{30}O_2$	242.4	6.10
10.	15.8677	2-Methyl-2,3-divinyloxirane		C7H10O	110.15	5.83
11.	15.943	Carbonic acid, dodecyl 2,2,2 -trichloroethyl ester		$C_{15}H_{27}Cl_3O_3$	361.73	5.70
12.	21.8837	Bis(3-methylbutan-2-yl) phtalate		$C_{18}H_{26}O_4$	306.39	7.76
13.	25.862	1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro		$C_{12}H_{17}NO_2$	207.27	1.96
14.	26.8104	Acetic acid, 2-[bis(methylthio) methylene]-1-phenylhydrazide		$C_6H_6N_2S_2$	170.26	3.64
16.	**26.9367**	Heptasiloxane,1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl		$C_{14}H_{42}O_6Si_7$	503.07	3.41

Key: PK=Peak, RT = Retention time

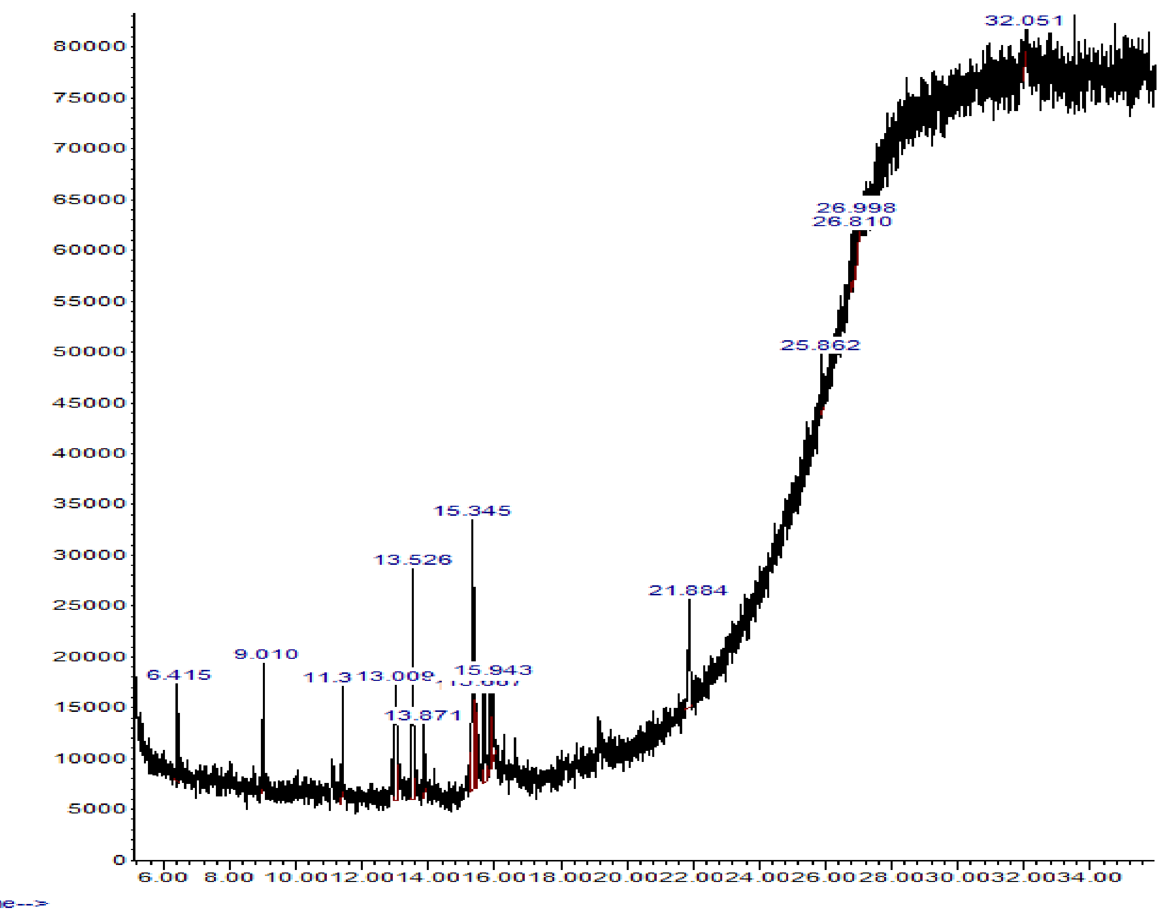

Figure 4.38: GC-MS profile of eluate B (**E_B**) from hexane fraction of methanol extract of *D.arborescens* roots

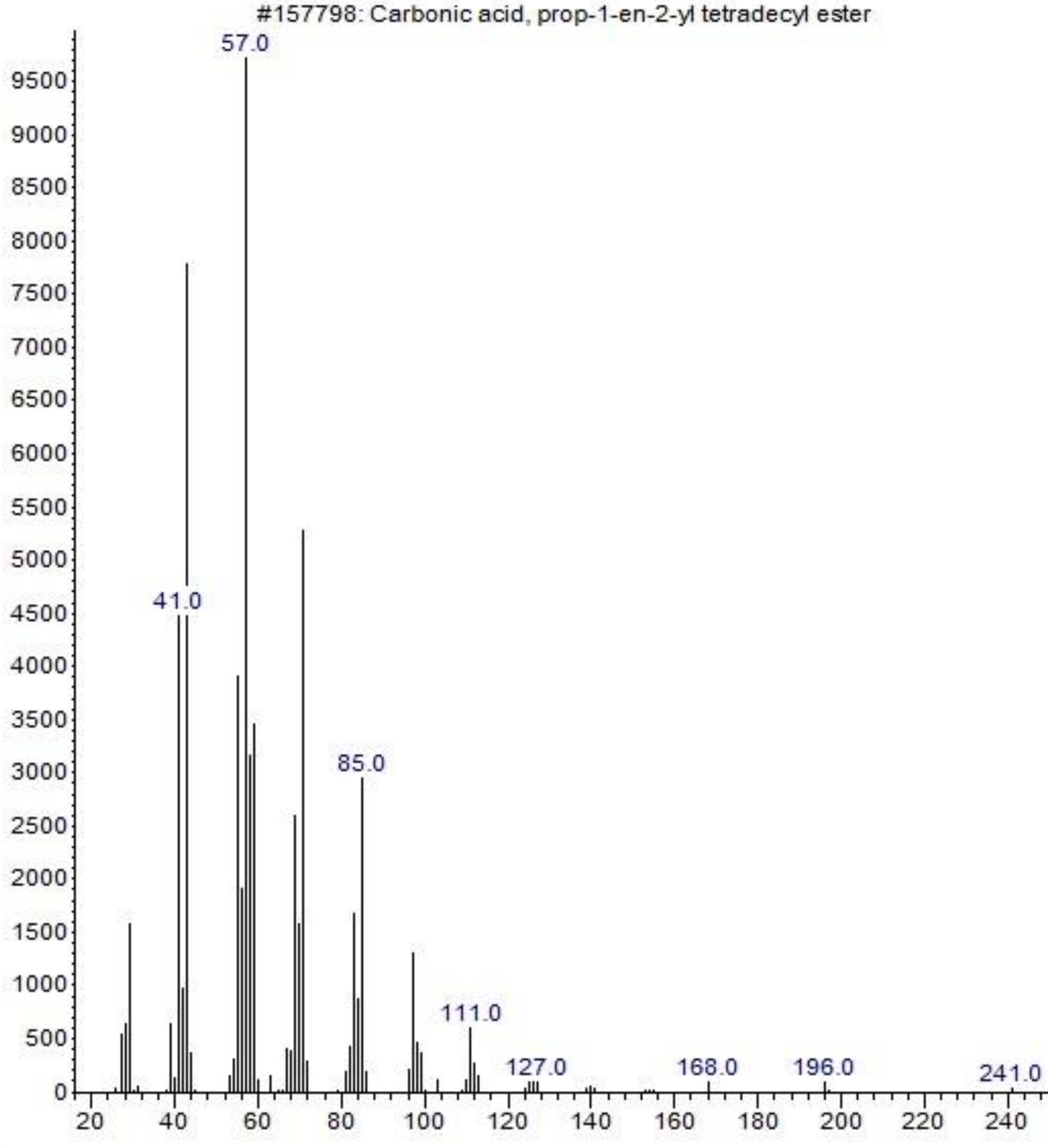

Figure 4.39: Mass spectrum of Carbonic acid prop-1- en – 2- yl tetradecyl ester

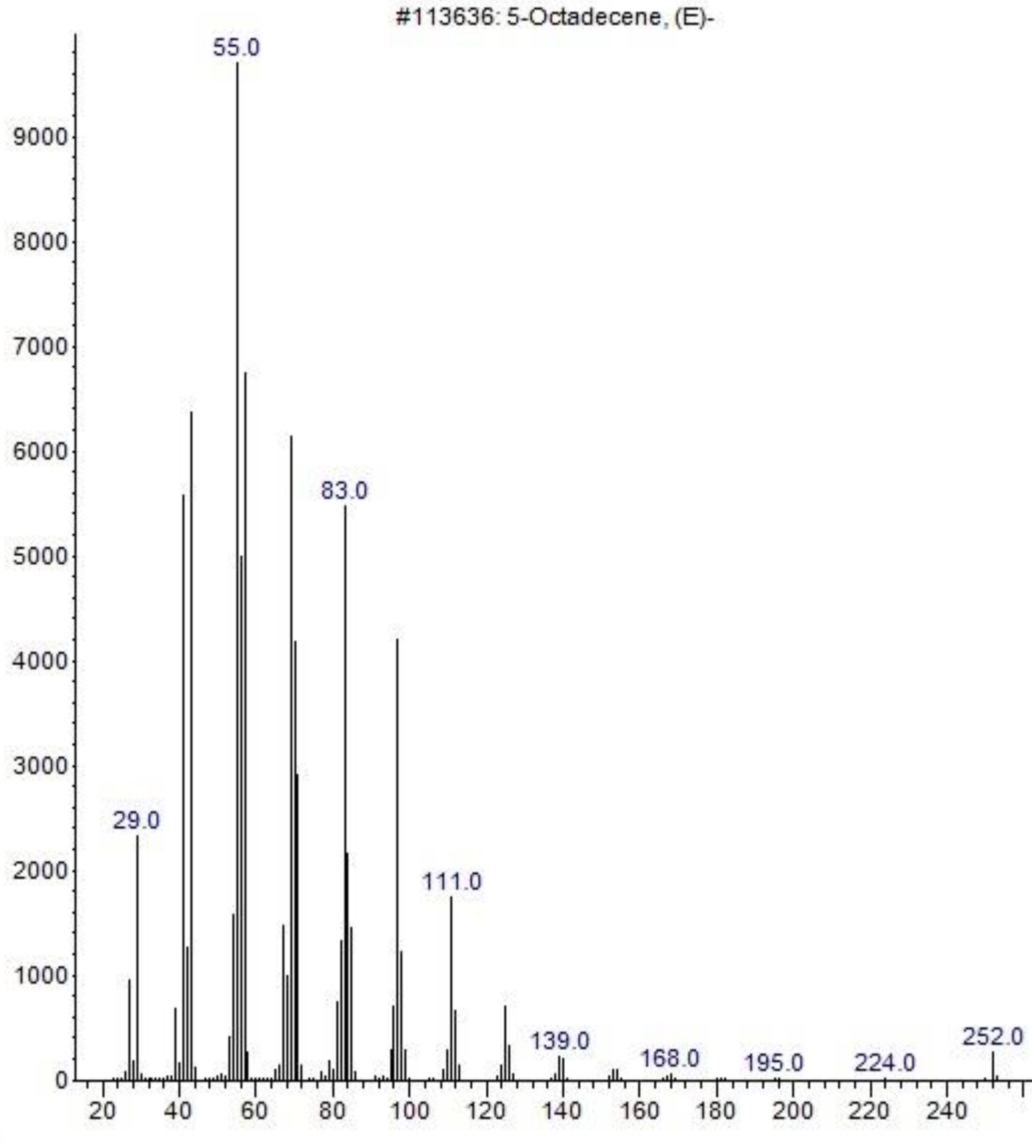

Figure 4.40: Mass spectrum of 5- Octadecene, (E)-

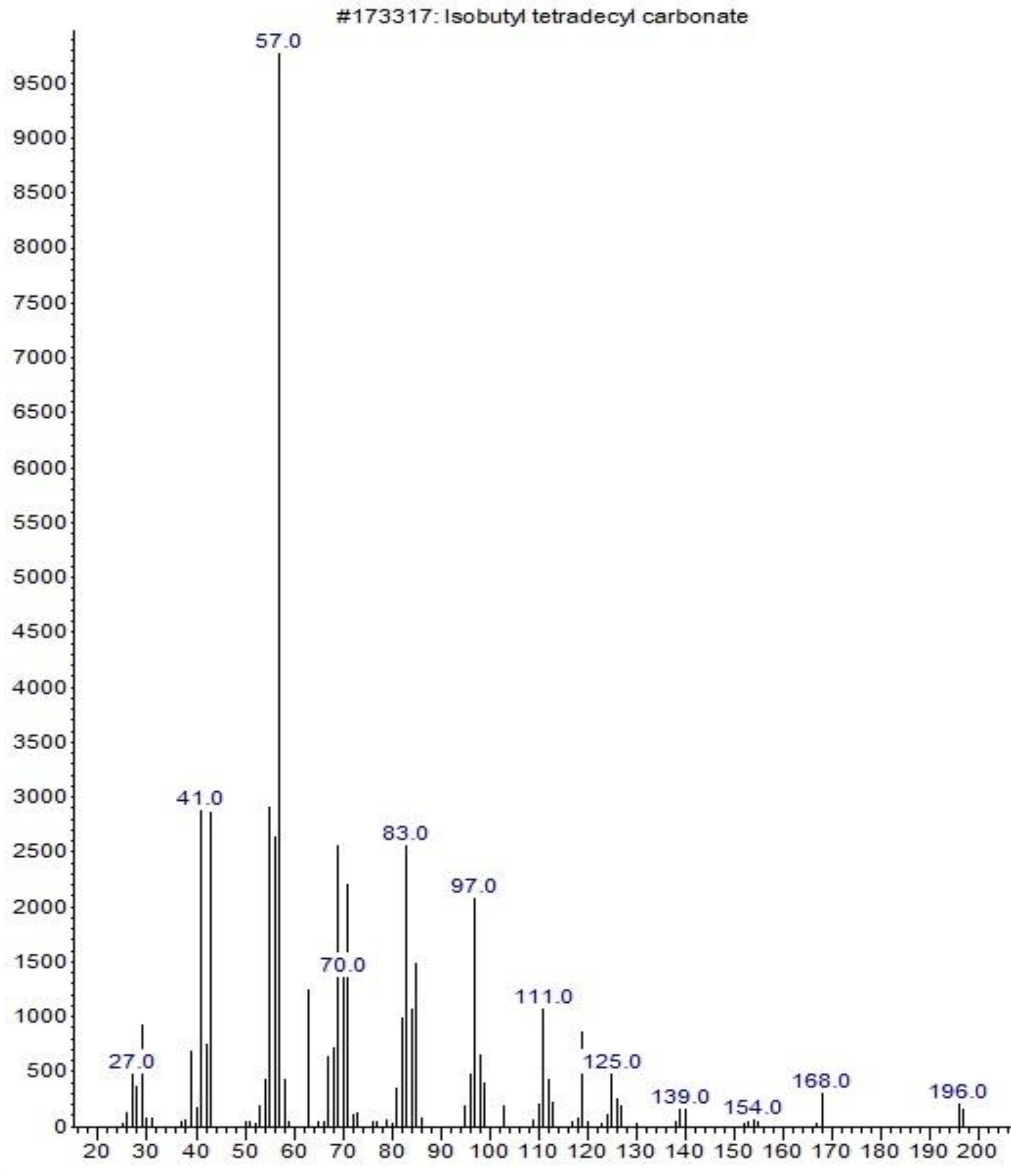

Figure 4.41: Mass spectrum of Isobutyl tetradecyl carbonate

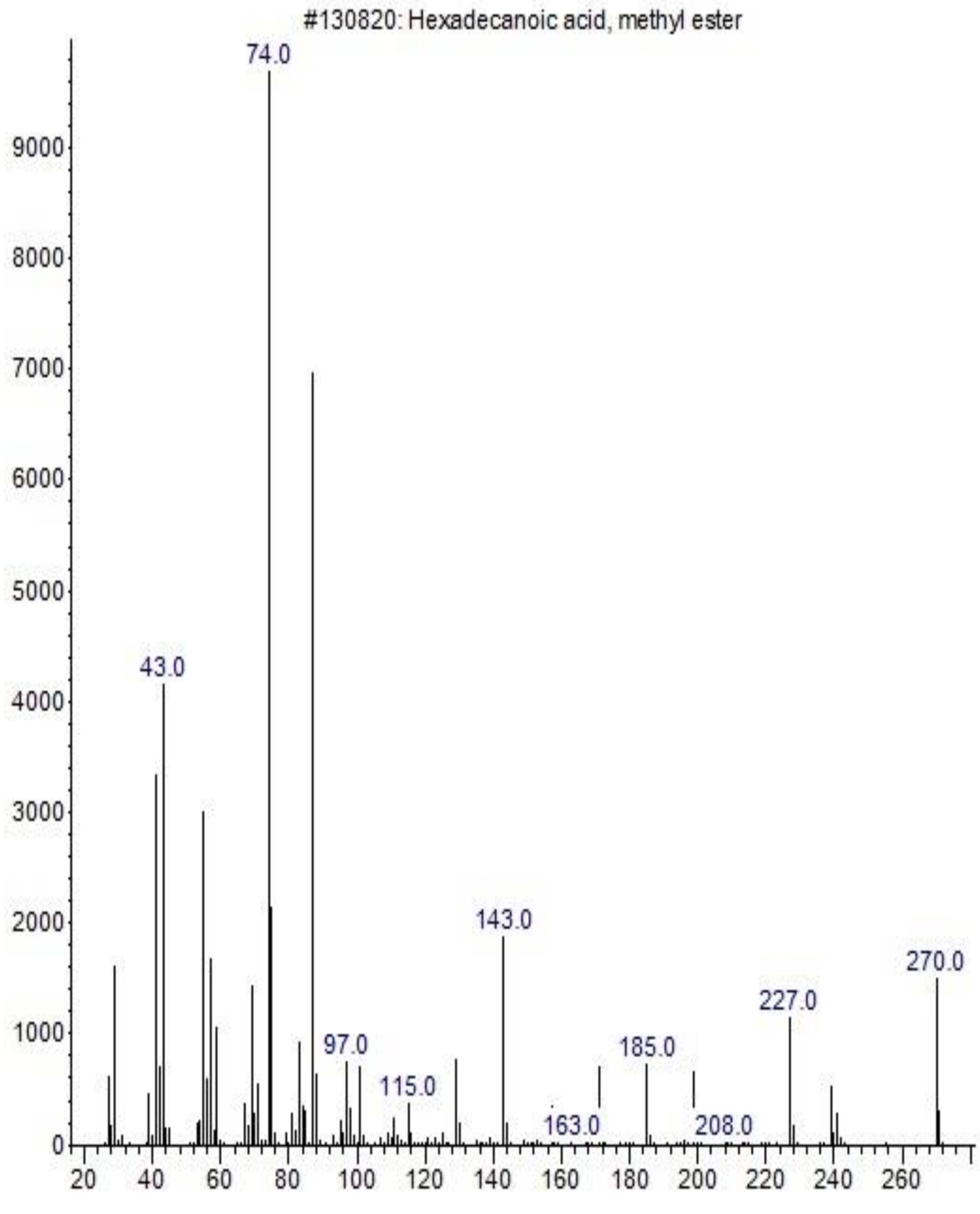

Figure 4.42: Mass spectrum of Hexadecanoic acid, methyl ester

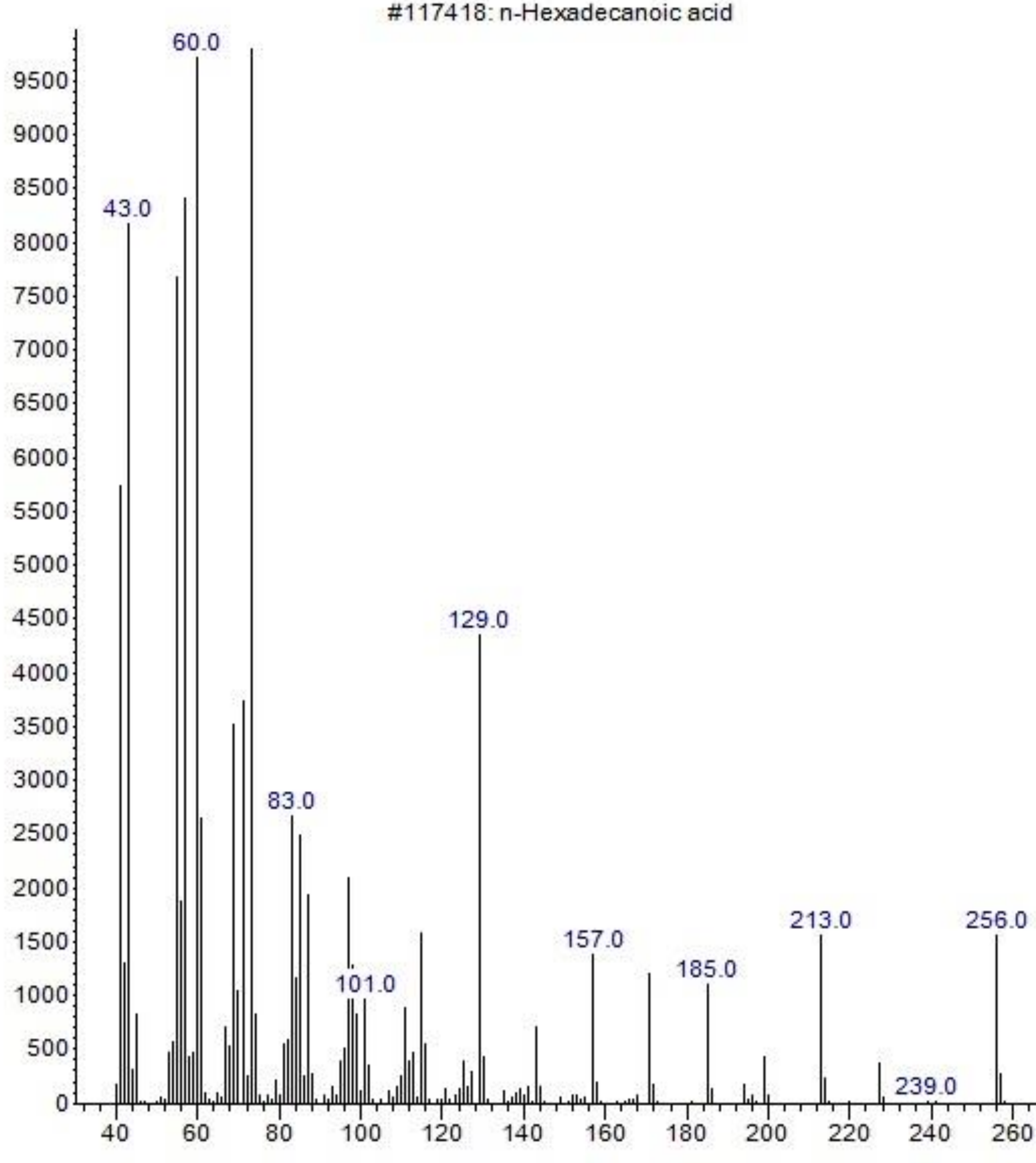

Figure 4.43: Mass spectrum of n-Hexadecanoic acid

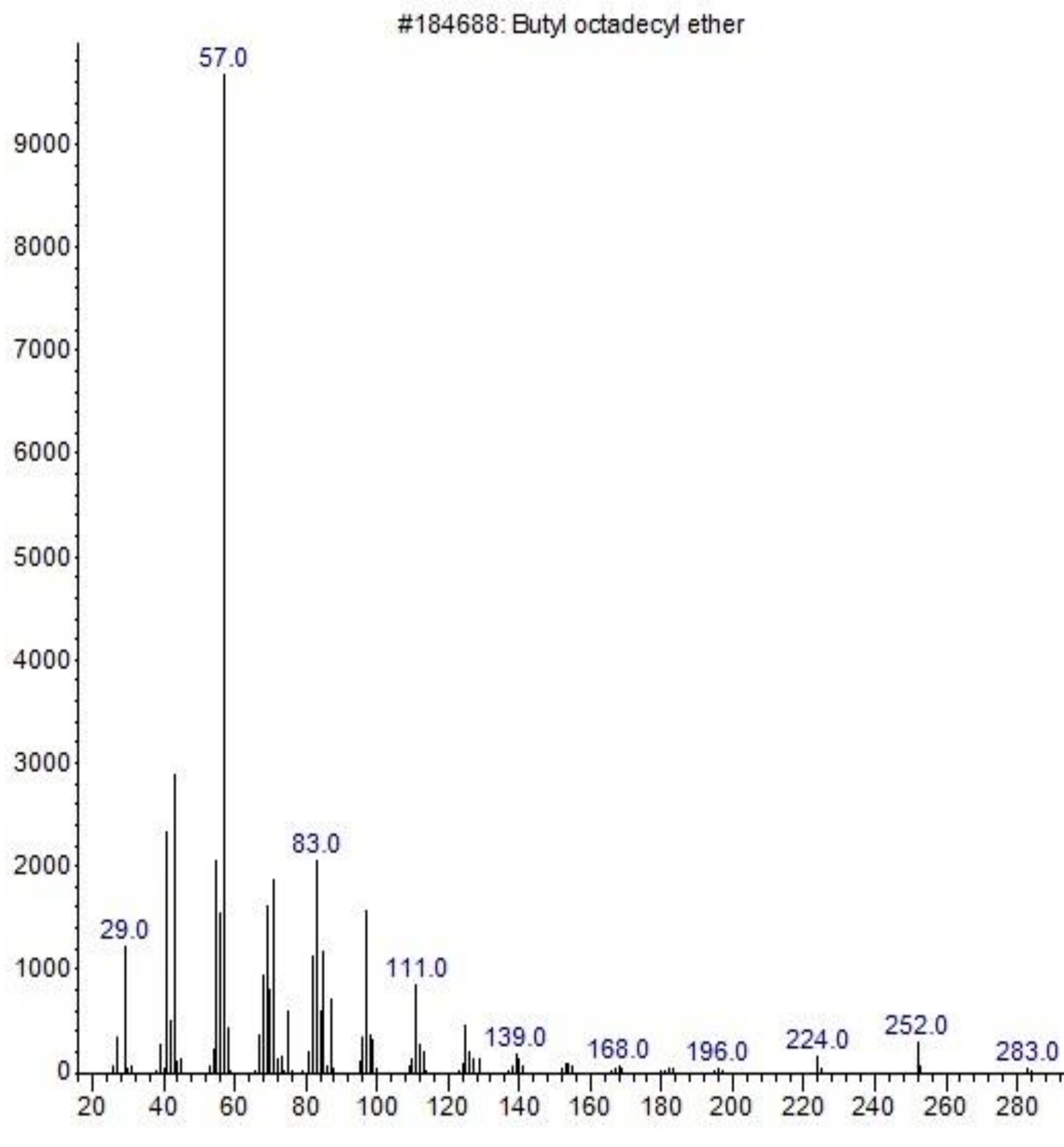

Figure 4.44: Mass spectrum of Butyl Octadecyl ether

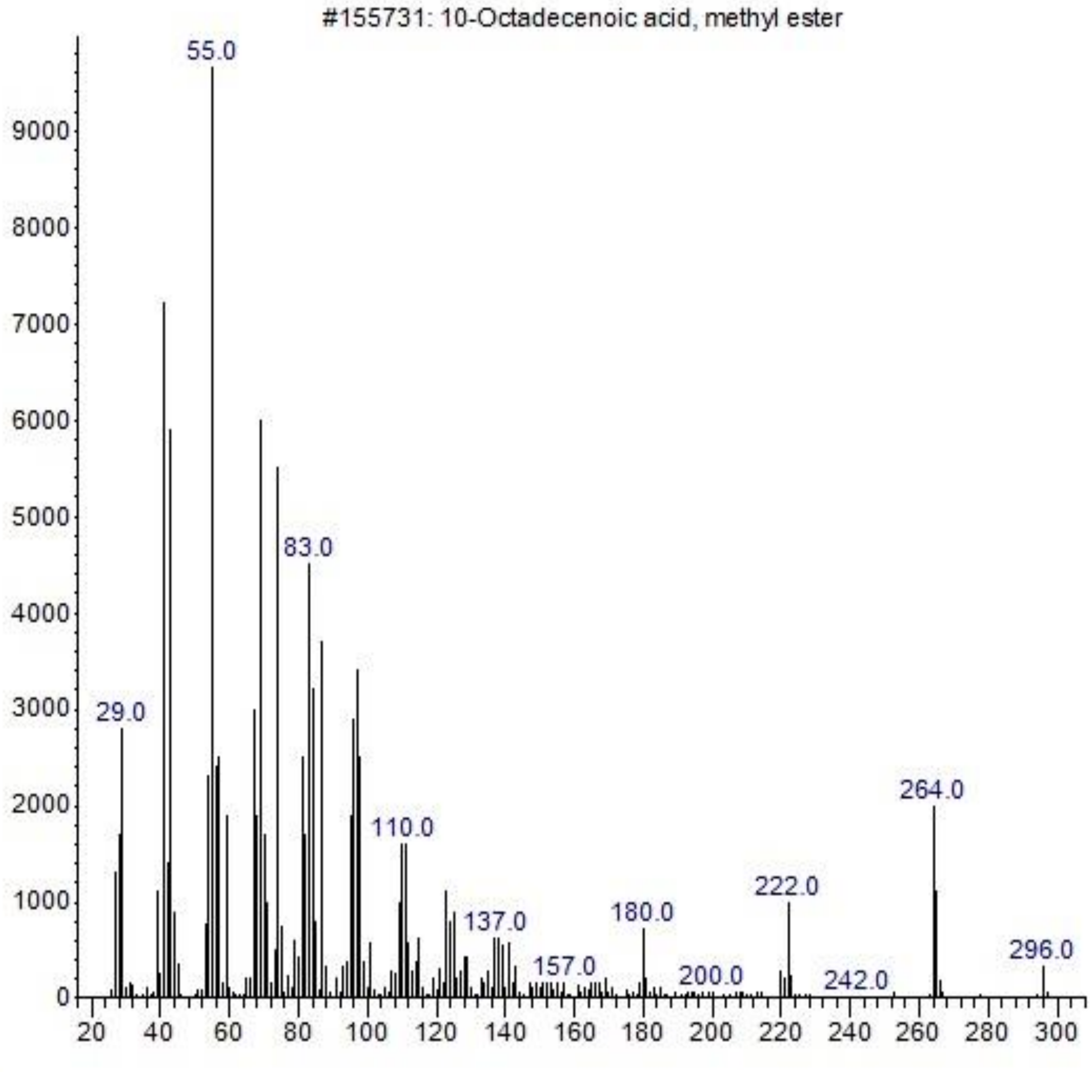

Figure 4.45: Mass spectrum of 10-Octadecenoic acid, methyl ester

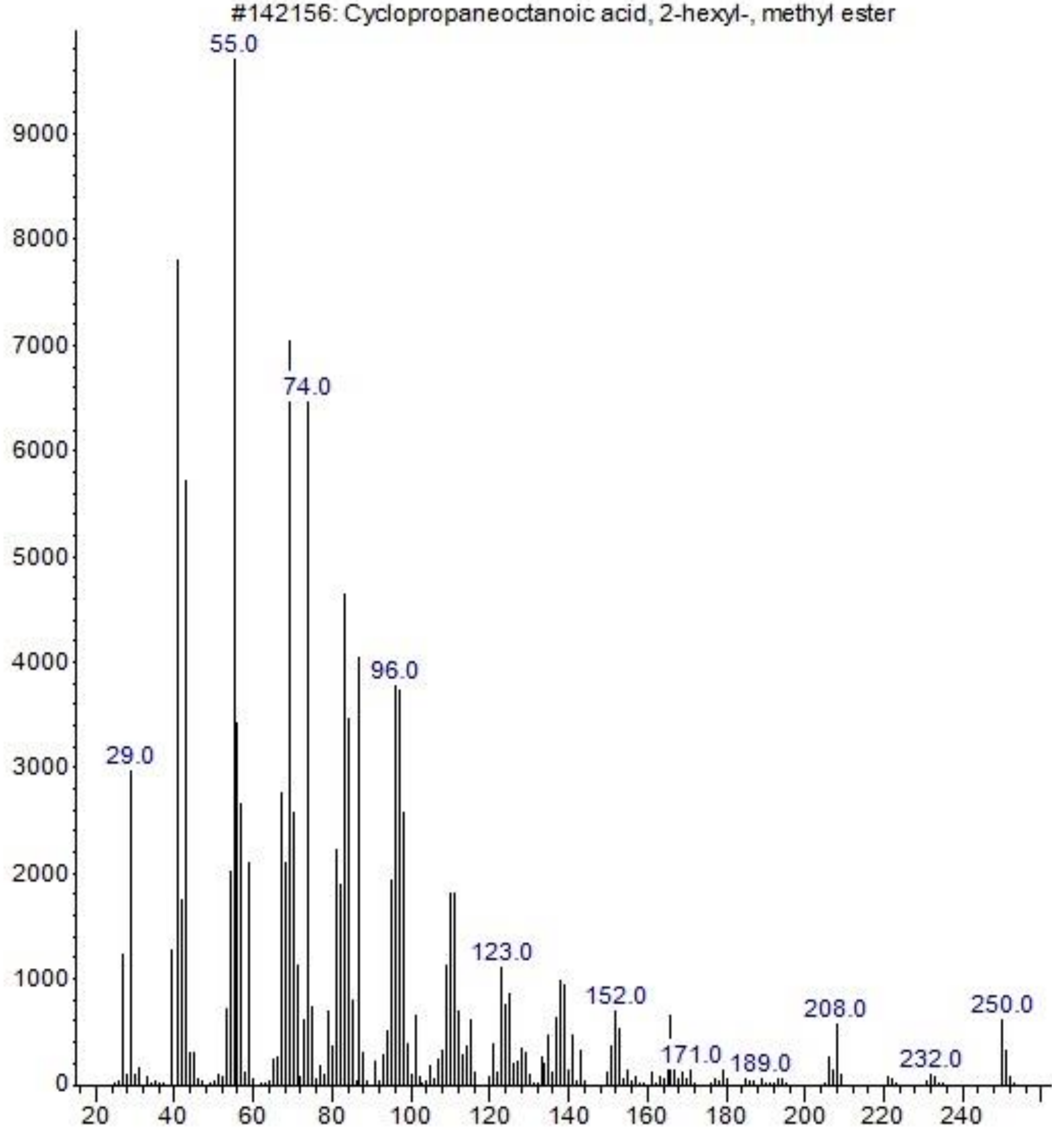

Figure 4.46: Mass spectrum of Cyclopropane Octanoic acid, 2-hexyl-, methyl ester

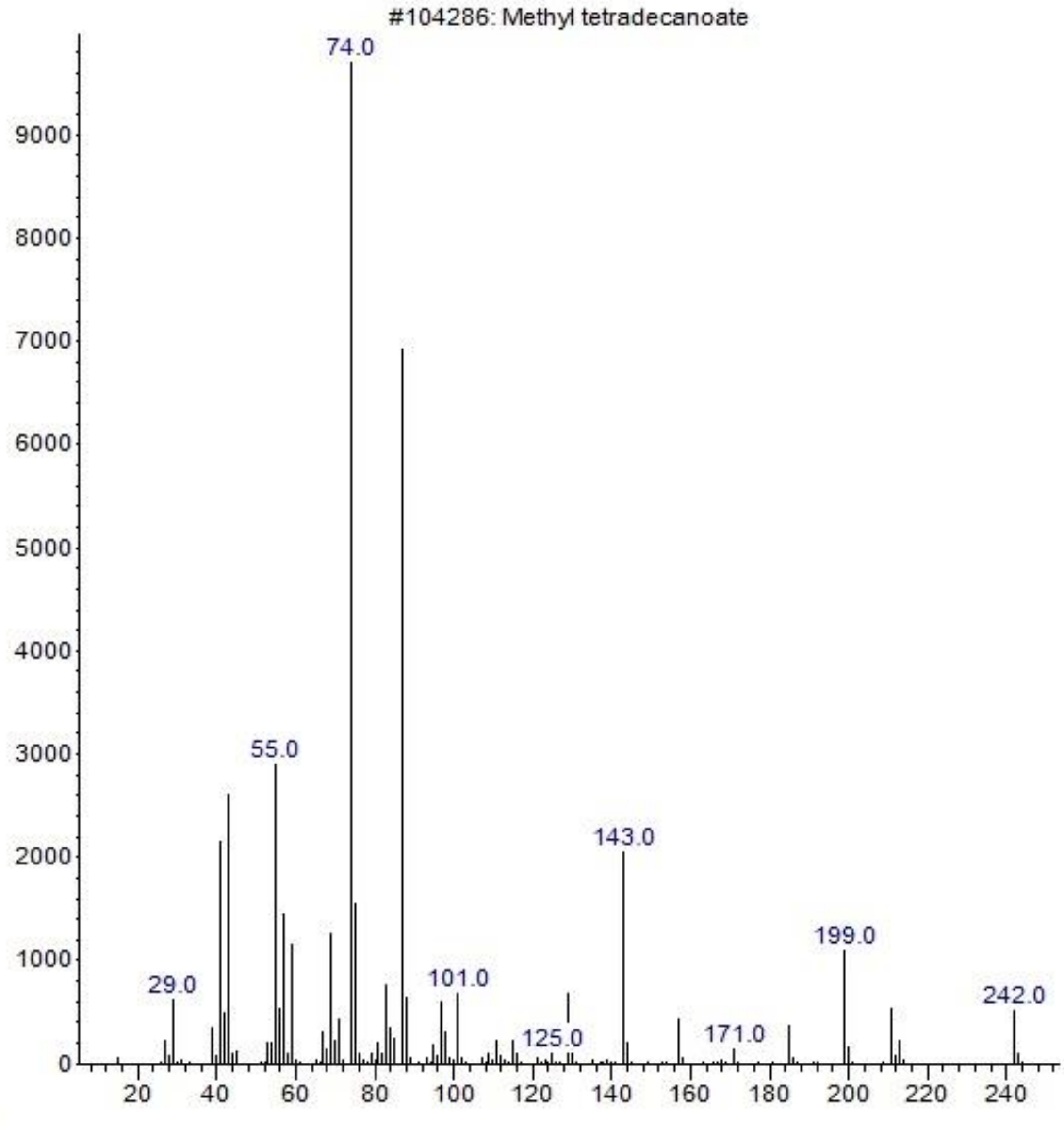

Figure 4.47: Mass spectrum of Methyl tetradecanoate

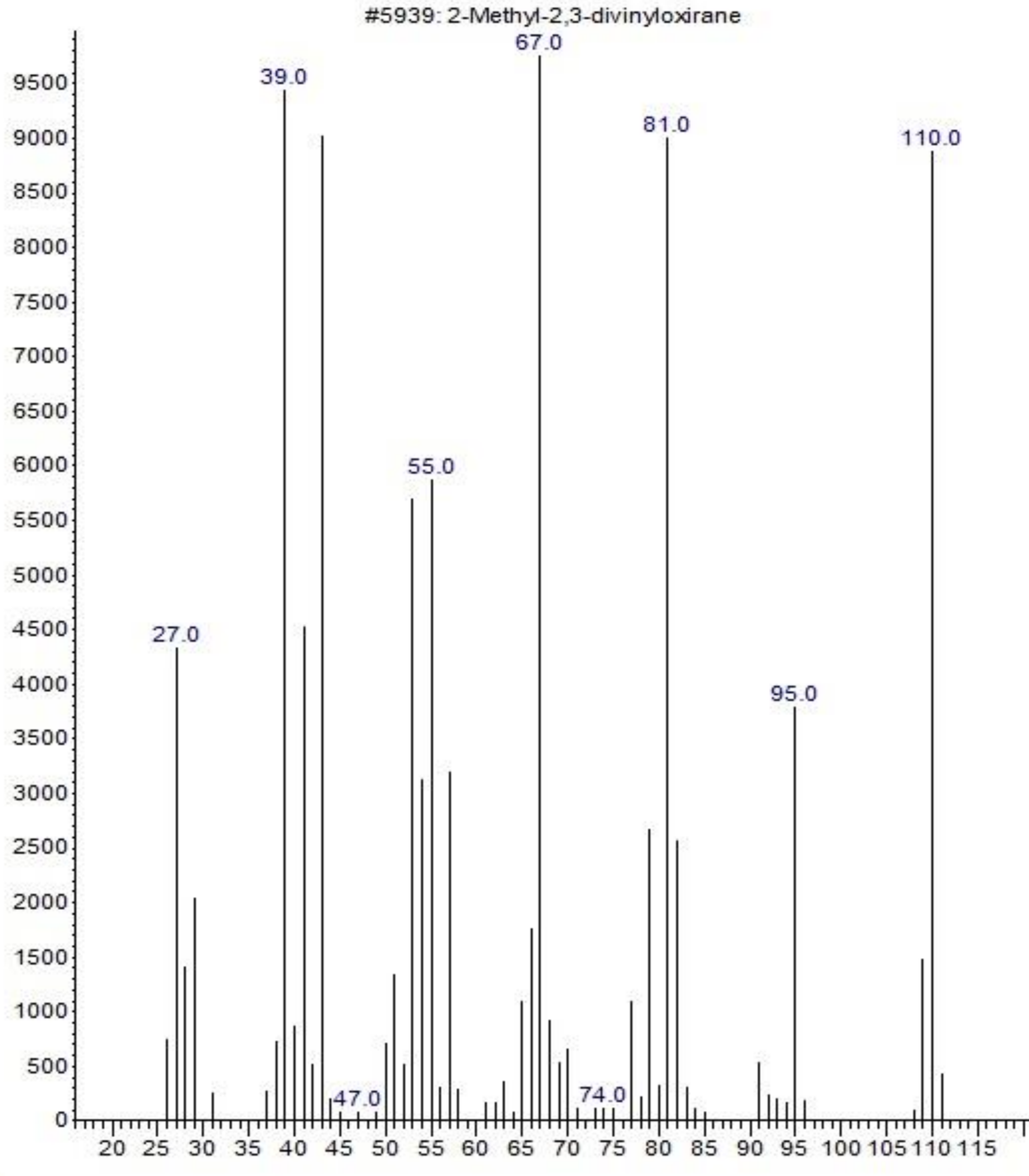

Figure 4.48: Mass spectrum of Methyl-2,3-divinyloxirane

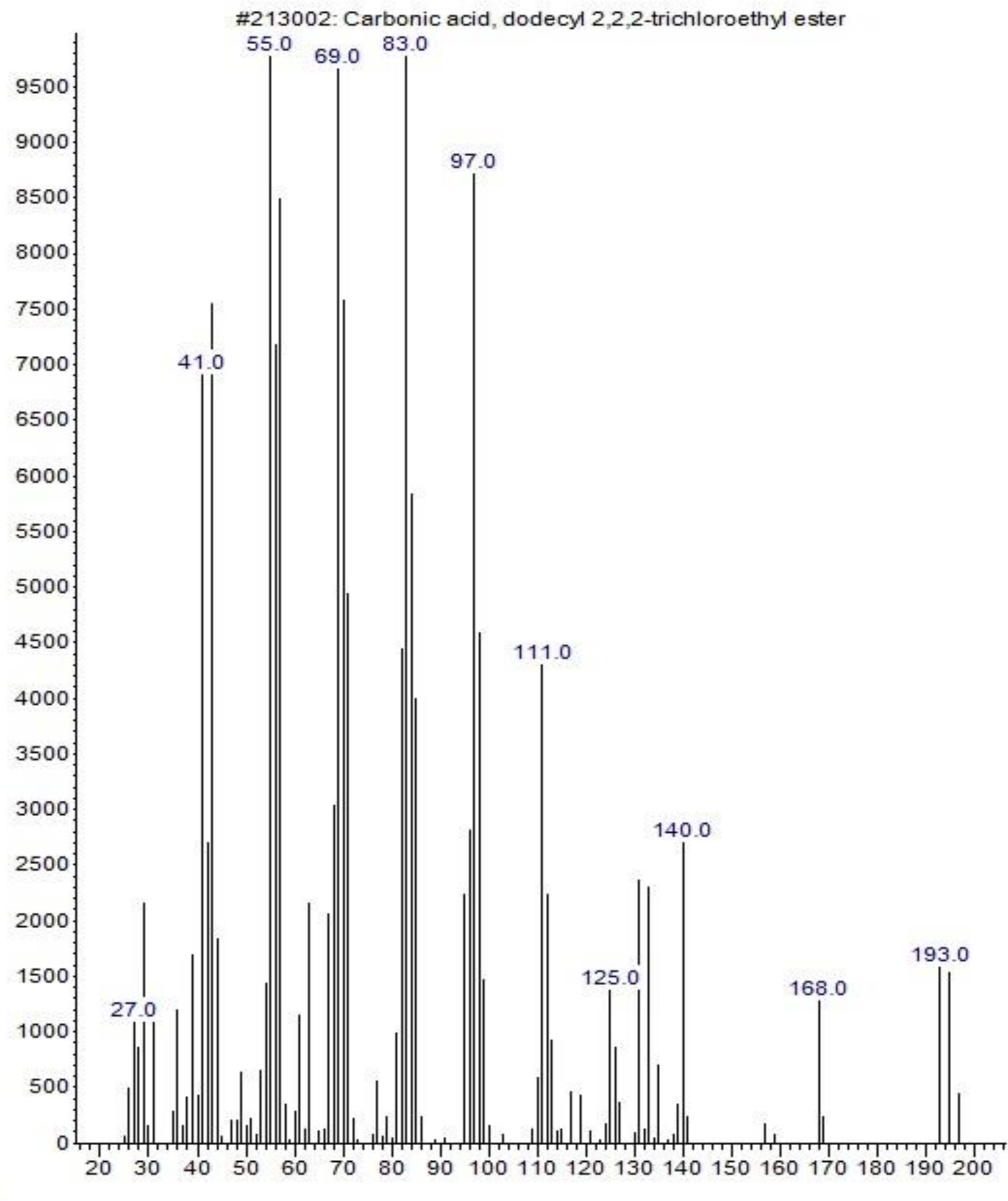

Figure 4.49: Mass spectrum of Carbonic acid, dodecyl 2,2,2- tricholoethyl ester

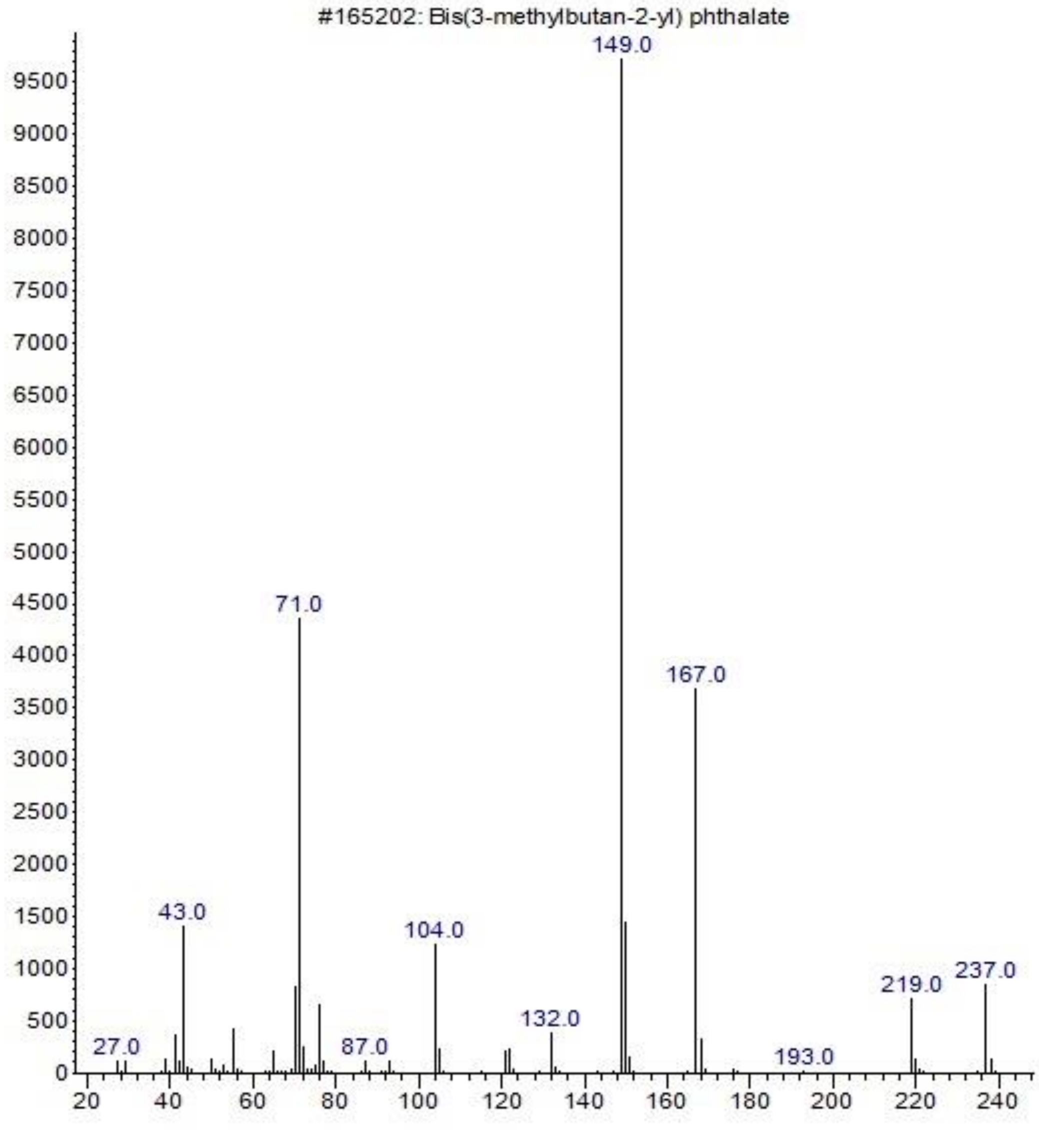

Figure 4.50: Mass spectrum of Bis(3-methylbutan-2-yl) phthalate

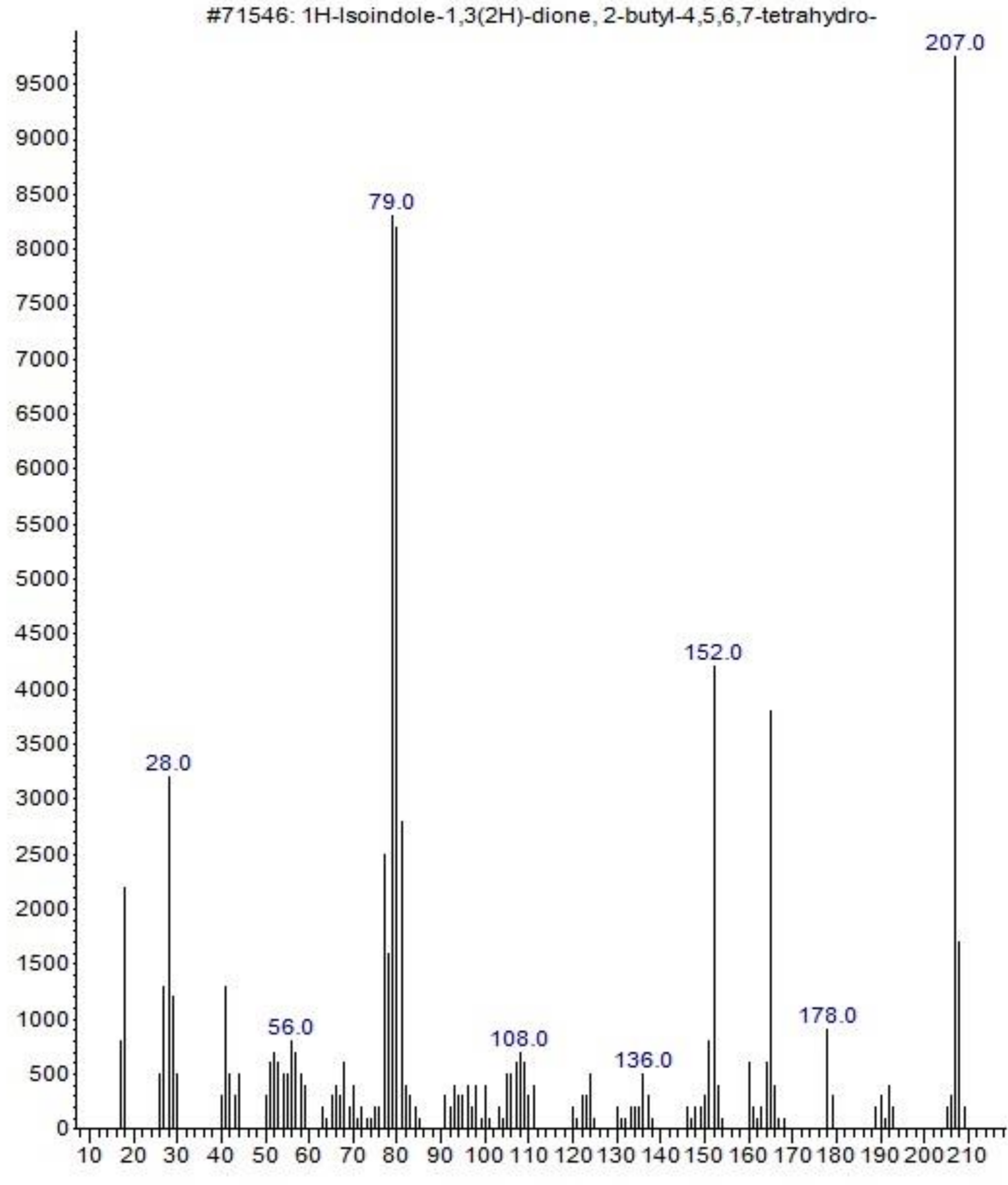

Figure 4.51: Mass spectrum of 1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro-

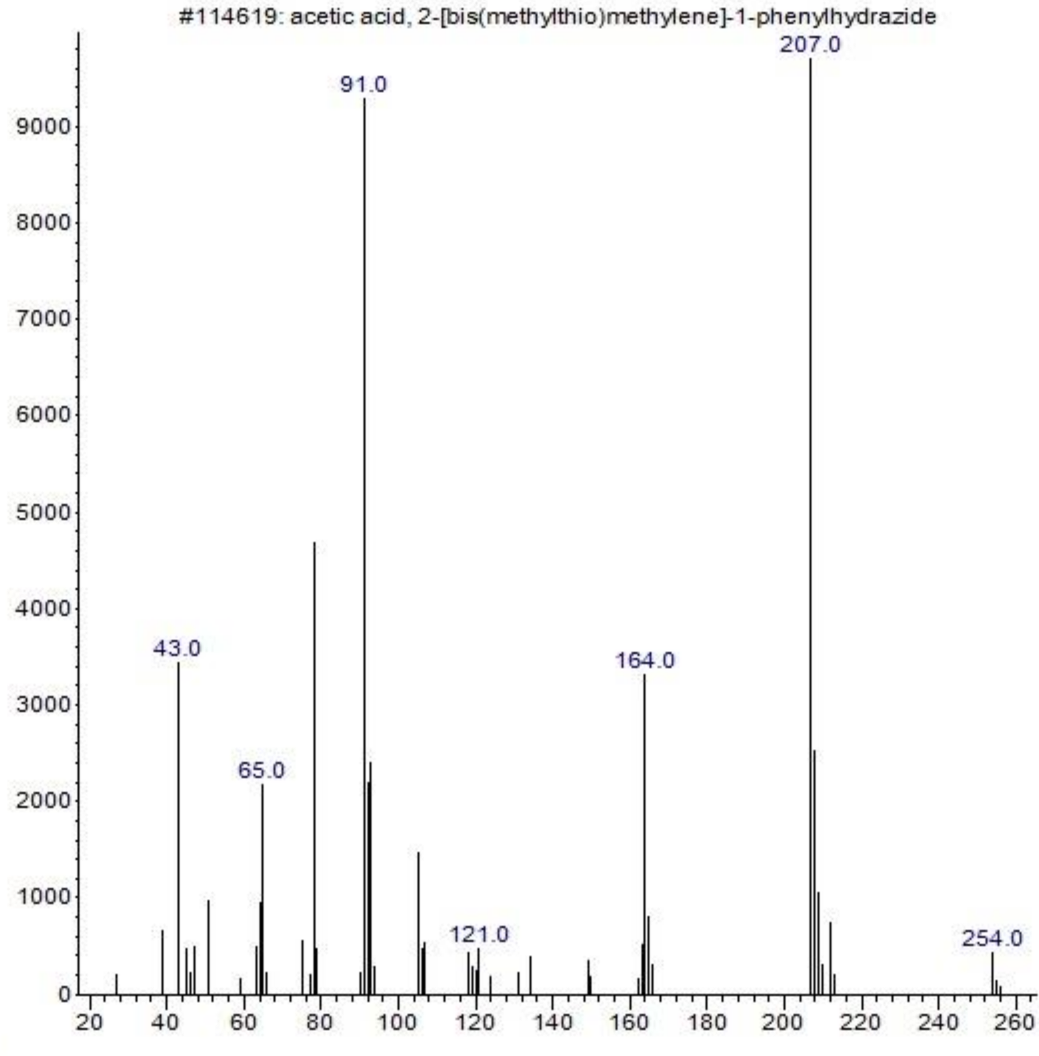

Figure 4.52: Mass spectrum of acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide

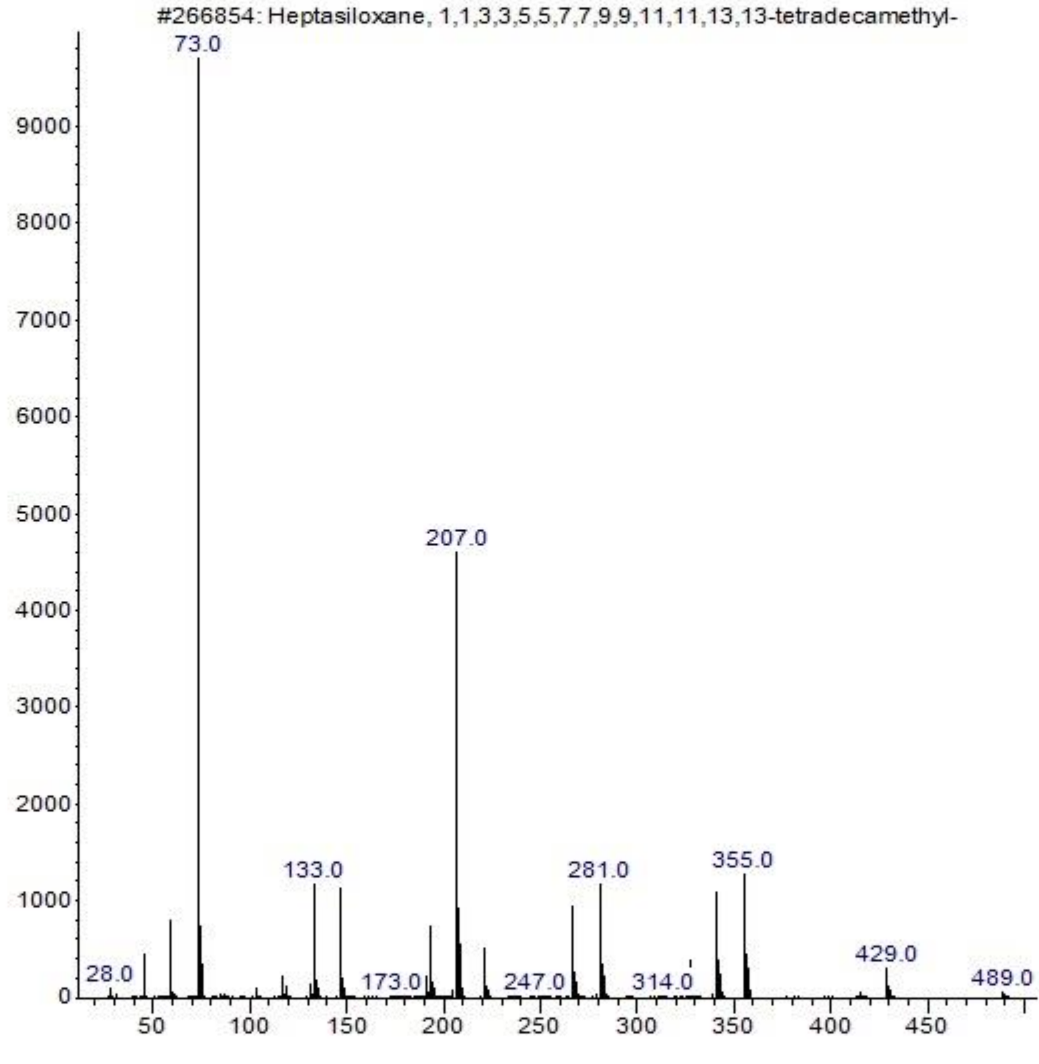

Figure 4.53: Mass spectrum of Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-

4.1.14 Molecular docking analysis of chemical compounds from GC-MS of eluate A (E_A) and eluate B (E_B) of hexane fraction of methanol extract of *D.arborescens* roots

Chemical compounds obtained from results of GCMS analysis were subjected to molecular docking, with some antimalarial protein targets, *Plasmodium berghei* lactate dehydrogenase and plasmepsin II. Hexane fraction of methanol extract of *D. arborescens* root compounds recorded varying degrees of binding affinities for the protein targets as shown by the change in Gibb's free energy (ΔG) (Table 4.32). For compounds present in eluate **A (E_A)**, the best binding affinity or docking scores (- 6.1 Kcal/mol and -6.6 Kcal/mol) was recorded for Bis (2-ethylhexyl) phthalate for the two protein targets. For compounds in eluate **B** (**E_B**), Bis(3-methylbutan-2-yl) phthalate had the best binding affinity, with docking scores of -6.2Kcal/mol and -6.0Kcal/mol for the two protein targets.

These docking scores were recorded to be close to those of the standard ligand, artesunate with binding affinity (docking scores) of -8.1Kcal/mol and -7.5Kcal/mol for the two protein targets, lactate dehydrogenase and plasmepsin II respectively. The protein residues that interacted with Bis(2-ethylhexyl) phthalate, Bis(3-methylbutan-2-yl) phthalate and Artesunate at the enzyme active site are summarized in Tables 4.34 and 4.35.

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of the compounds with the highest binding affinities also suggest that these compounds are not toxic compared to the standard antimalarial drug used in this study (Table 4.36).

Table 4.32: Binding affinities of chemical compounds in eluate A (E_A) of hexane fraction of methanol extract of *D.arborescens* roots for the two antimalarial protein targets.

S/N	Chemical compounds in eluate A (E_A)	Binding affinity (Kcal/mol)	
		1OC4	**1SME**
1.	Propane, 1,2-dichloro-	-3.2	-2.7
2.	2-Pentyn-1-ol	-4.1	-4.0
3.	Dichloroacetic acid, tridecyl ester	-4.5	-3.8
4.	1-Octadecene	-4.1	-3.8
5.	Hexadecanoic acid, methyl ester	-4.7	-3.6
6.	n-Hexadecanoic acid	-4.6	-4.5
7.	9,17-Octadecadienal, (Z)-	-4.3	-3.9
8.	cis-13-Octadecenoic acid, methyl ester	-4.5	-5.9
9.	6-Octadecenoic acid, methyl ester, (Z)-	-4.2	-5.1
10.	Heptadecanoic acid, 16-methyl-, methyl ester	-4.4	-5.6
11.	**Bis(2-ethylhexyl) phthalate**	**-6.1**	**-6.6**
12.	**Artesunate (control)**	**-8.1**	**-7.5**

Table 4.33: Binding affinities of chemical compounds in eluate B (E_B) of hexane fraction of methanol extract of *D.arborescens* roots for the two antimalarial protein targets.

S/N	Chemical compounds in eluate B (E_B)	Binding energy (Kcal/mol)	
		1OC4	**1SME**
1.	Carbonic acid, prop-1-en-2-yl tetradecyl ester	-4.5	-5.6
2.	5-Octadecene, (E)-	-4.2	-3.6
3.	Isobutyl tetradecyl carbonate	-4.7	-5.3
4.	Hexadecanoic acid, methyl ester	-4.4	-4.2
5.	n-Hexadecanoic acid	-4.5	-4.4
6.	Butyl hexadecyl ether	-4.1	-4.9
7.	10-Octadecenoic acid, methyl ester	-5.0	-4.3
8.	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	-5.0	-5.4
9.	Tetradecanoic acid, 12-methyl-, methyl ester	-4.8	-5.2
10.	2-Methyl-2,3-divinyloxirane	-4.3	-4.3
11.	Carbonic acid, dodecyl 2,2,2-trichloroethyl ester	-4.6	-4.2
12.	**Bis(3-methylbutan-2-yl) phthalate**	**-6.2**	**-6.0**
13.	1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro-	-5.6	-5.7
14.	acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide	-4.9	-5.0
15.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	0.0	0.0
16.	**Artesunate**	**-8.1**	**-7.5**

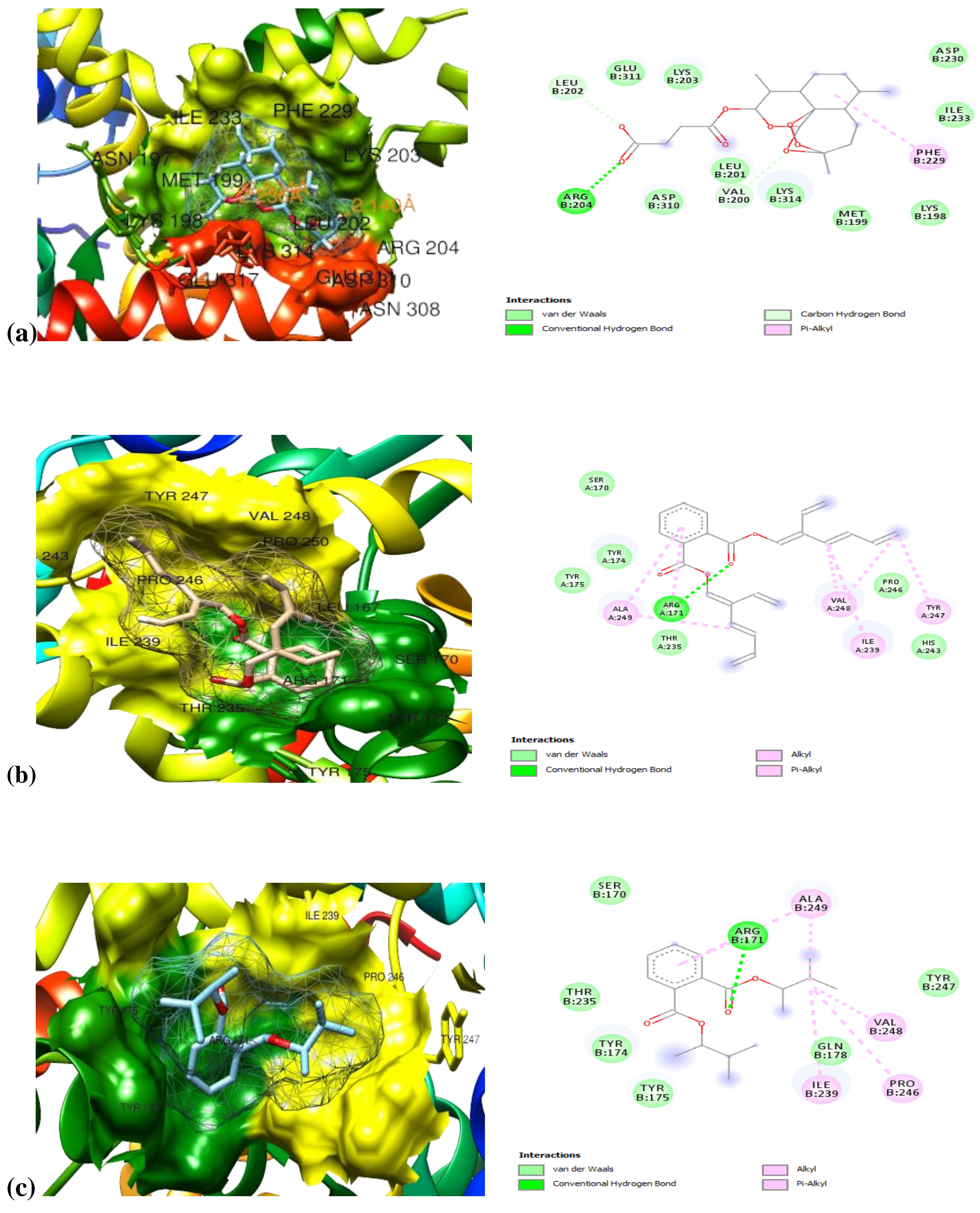

Figure 4.54: 3D (left) and 2D (right) view of (a) Artesunate (b) Bis(2-ethylhexyl) phthalate (c) Bis(3-methylbutan-2-yl) phthalate interacting with Lactate dehydrogenase (1OC4)

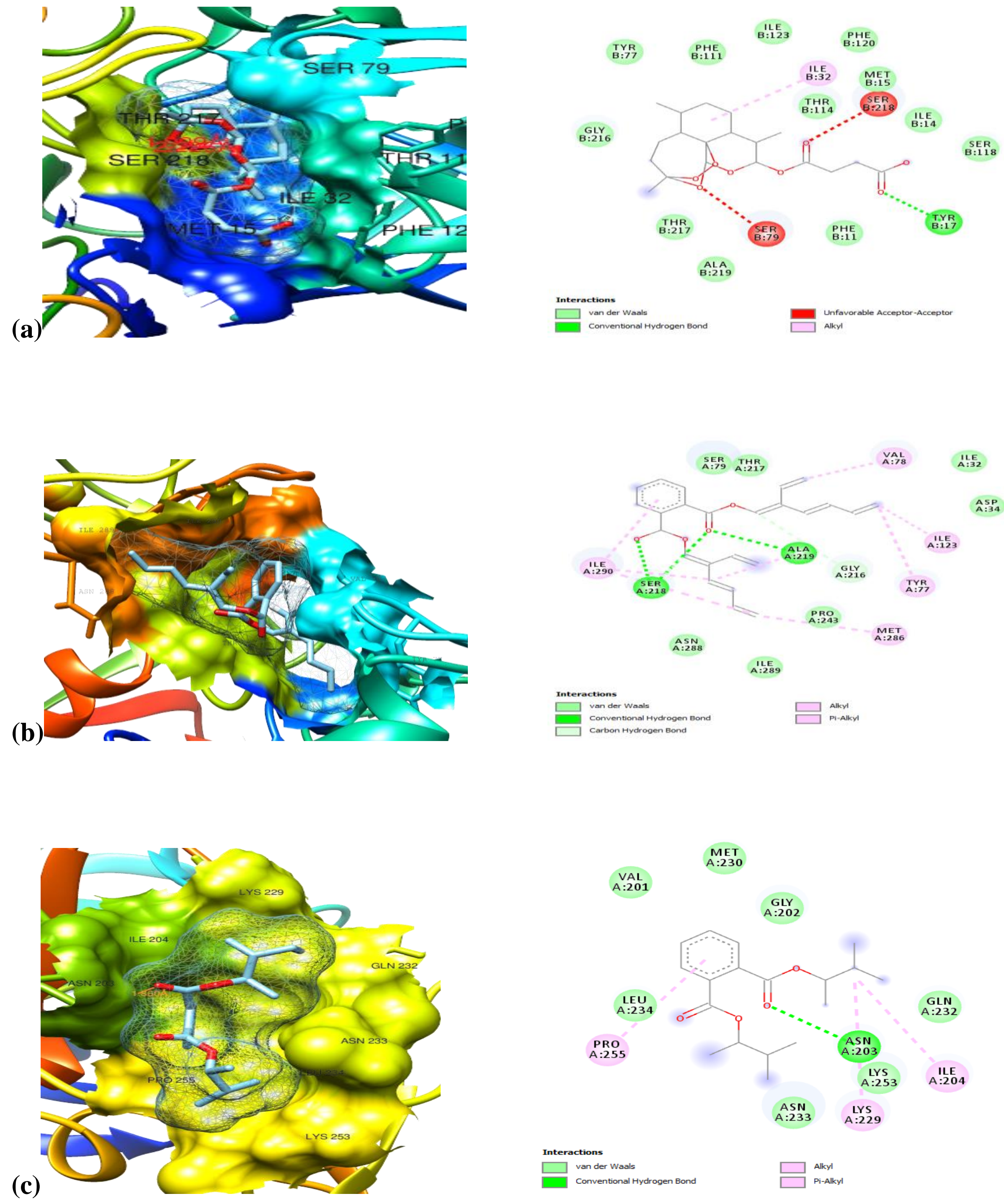

Figure 4.55: 3D and 2D view of (a) Artesunate (b) Bis(2-ethylhexyl) phthalate (c) Bis(3-methylbutan-2-yl) phthalate interacting with plasmepsin II (1SME)

Table 4.34: Protein-residue interactions with Artesunate, Bis(2-ethylhexyl) phthalate and Bis(3- methylbutan-2-yl) phthalate for lactate dehydrogenase

Compound	Van der Waals	Hydrogen bond	Carbon hydrogen bond	Pi-Akyl	Akyl
Bis(2-ethylhexyl) phthalate	SER170 TYR174 TYR175 TYR235 PRO246 HIS243	ARG171	-	ALA249	VAL248 TYR247 ILE239
Bis(3-methylbutan-2-yl) phthalate	SER170 THR235 TYR174 TYR175 GLN178 TYR247	ARG171	-	ILE239	ALA249 VAL248 PRO246
Artesunate	GLU311 LYS203 ASP230 ILE233 ASP310 LEU201 LYS314 MET199 LYS198	ARG204	LEU202 VAL200	PHE229	-

The chemical compounds interacted with the residues using similar interactive forces. They formed hydrogen bonds with ARG171 and with other similar interactions, though at different residues. This is an indication that the mechanism of action of the compounds on the target is similar, but different from the control drug.

Table 4.35: Protein-residue interactions with Artesunate, Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate for plasmepsin II

Compound	Van der Waals	Hydrogen bond	Carbon hydrogen bond	Pi-Akyl	Akyl	Unfavourable Acceptor-acceptor
Bis(2-ethylhexyl) phthalate	SER79 THR217 ILE32 ASP34	SER218 ALA219	ASN288 ILE289 PRO243 GLY216	ILE290 MET286	VAL78 ILE123 TYR77	-
Bis(3-methylbutan-2-yl) phthalate	VAL201 MET230 GLY202 LEU234 ASN233 LYS253 GLN232	ASN203	-	LYS229 ILE204	PRO255	-
Artesunate	TYR77 PHE111 ILE123 PHE120 THR114 MET15 ILE14 SERII8 PHE11 GLY216 THR217 ALA219	TYR17	-	-	ILE32	SER79 SER218

The interactions of the compounds and the control drug occurred at different residues indicating dissimilar mechanisms of action by them.

The pharmacokinetic and pharmacodynamic properties of Bis(2-ethylhexyl) phthalate, Bis(3-methylbutan-2-yl) phthalate and artesunate revealed by their ADMET properties are summarized in Table 4.36. The drug likeliness of Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate was assecced from Lipinski's rule of five (Lipinski, 2016). Any good drug candidate should not violate more than one of the rules. The values were compared with those obtained for artesunate.

Table 4.36: ADMET Properties of Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate compared to artesunate

Compounds in eluate A						PARAMETERS				
	Acute Oral Toxicity (c)	**Blood Brain Barrier**	**Caco-2**	**Carcino genicity (binary)**	**Hepato toxicity**	**Human Intestinal Absorption**	**Human oral bio availabiliy**	**Acute Oral toxicity**	**Plasma protein binding**	**Water solubility**
Bis(2-ethylhexyl) phthalate	IV	+	+	-	-	+	+	1.692999	0.978868	-628073
Bis(3-methylbutan-2-yl) phthalate	IV	+	+	-	-	+	+	1.917456	0.965625	-3.34289
Artesunate	IV	+	-	-	-	+	+	3.44439	0.996128	-4.72666

4.2 Discussion

Extraction yield (% w/w) is a measure of the efficiency of a solvent to extract particular components from the original material. It is usually defined as the amount of solid extract recovered in mass compared to the initial amount of plant material. Aqueous (water) and methanol solvents were used for extraction in this study in accordance with folkloric practice. Percentage yield of extract from methanol and aqueous solvent varied across the plant materials. Extract yield (% of dry wt) from the studied plants was higher with the organic solvent (methanol) than with the aqueous solvent. This agrees with the findings of Wilcox, Graz, Falquet, Diakite, Giani and Diallo (2014) that phytochemicals in plants are more soluble in organic solvents. The difference in yield may be attributed to the polarity of the extracted compounds as reported by Pareck, Jadeja and Chanda, (2015). The low polarity of methanol was probably accountable for the high yield of extracts because both polar and non polar compounds were extracted. Yield of extract was low compared with the ground plant material used for extraction. This may be due to the difference in methods of extraction (maceration and soxhlet) used in the study. Maceration was reported to give low yield of extract compared to soxhlet and other methods of extraction (Ibrahim, Bolaji, Abdulrahman, Ilyas & Habib, 2005). It was chosen as one of the extraction methods in this research because it required no heating, thus preserving the biologically active components. In this study, methanol solvent extracted more of the bioactive compounds than the aqueous solvent suggesting that crude powder extracts obtained from *C. dependens* and *D. arborescens* could be affected by the extracting solvents. These findings are in agreement with the reports of Do, Angkawijaya, Phuong, Huynh, Soetaredjo and Ismadji, (2014) on *Limnophila aromatic*. The results also agreed with Do *et al*. (2014) that increasing the water concentration in the solvent enhances extraction yield. It is also in tandem with findings of Anwar & Przybylski (2012) who

reported that aqueous methanol yielded higher amount of extractable solids in barley and flax seeds. The variation can be attributed to difference in solubility of the various compounds in the samples. Generally, these findings suggested that most of the compounds in the two plants are dissolveable in methanol and a mixture of 20% (v/v) water with methanol was a good choice for obtaining better extractable solids from these plants.

Phytochemical screening is usually used to determine the general 'finger-print' or chemical profile of a particular plant extract (Bandaranayake, 2006). Preliminary phytochemical screening is often based on observation of change in colour to determine the presence of some plant constituents. Preliminary phytochemical screening was carried out on the various extracts to ascertain presence of compounds with antimalarial properties were in the plant extracts which will encourage further pharmacological investigations. Aremu, (2009) reported that there is a direct correlation between phytochemical constituents present in a plant and the therapeutic activity of such plant. Thus, results obtained from phytochemical screening in this study, provided preliminary information on some compounds known for antimalarial activities.

Results obtained from the phytochemical screening of the leaves, stem and roots of *C. dependens* and *D. arborescens* plants revealed presence of tannins, saponins, flavanoids, alkaloids, cardiac glycosides, terpenoids, steroids, phenols, oxalate and phytate. These phytochemicals were either low (+), moderate (++), high (+++) or not detected (ND). Results obtained showed that most of the screened phytochemicals were present giving credence to use of these plants in treatment of various ailments, especially malaria.

These phytochemical constituents have earlier been reported to have varying degrees of therapeutic activities (Badam, Bedekar, Sonawane, & Joshi 2012; Gupta and Tandon, 2014). Plants

have also been reported to have medicinal properties due to the presence of these rich bioactive compounds (Lewis & Ausubel, 2016). A complex mixture of these various phytochemicals confers on plants their therapeutic properties and antioxidant activities (Cartea, Francisco, Soengas & Velasco, 2011).

From results of quantitative phytochemical screening, tannins were high (31.24±0.03%) in the methanol extract of *D.arborescens* leaves. This phytochemical was equally high in the aqueous extract of the leaves of this plant. Presence of this phytochemical may be responsible for the antimalarial and antioxidant properties of the two plants. Tannins are polyphenols and are significant as a result of their physiological potentials. They are known for their anticancer activities; they also play a vital role in the treatment of inflamed and ulcerated tissues (Aleto & Adeogun 2005). Tannins also have antioxidant, anti-inflammatory, antitumor, antibacterial, antiviral, antimicrobial, antidiarrheal, antimalarial and antihaemorrhoid, properties (Lin, Hsu & Lin, 2011; Luthar, 2012; Vattern, Ghaedian & Shetty, 2015; Mori, Nishino, Enoki & Tawata, 2017). Terpenoids were present in aqueous and methanol extracts of the leaves, stem and roots of the two plants. This class of phytochemicals have biological activities and are used in fighting diseases such as malaria, inflammation, cancer etc (Abdul-Fadl, El-Badry & Ammar 2011; Sunita, Das, Singh, Marina, Carola & Cesar, 2017). Alkaloids were present in all the extracts but their concentrations ranged from moderate to low. The highest concentration (8.95±0.05%) was obtained from methanol extract of *D. arborescens* roots. This phytochemical was however not detected in the aqueous extract of the stem of *C.dependens* plant. Alkaloids are one of the most significant biologically active compounds in plants due to their therapeutic effects. Presence of alkaloids brings about some physiological changes in organisms, such as antimalarial, antifungal, antibacterial (Stary, 1998; Okwu & Okwu, 2004), anticancer, antioxidant and stimulating activities

(Ross & Brain, 2007) as well as analgesic activities (Stray, 1998). Alkaloids equally exhibit analgesic, antibacterial and antimalarial properties (Nyarko and Addy, 2009). They are a major class of compounds which possess antimalarial activity, and one of the oldest and important antimalarial drugs, quinine, belongs to this group (Dharani, Yenesew & Baide, 2008). Presence of alkaloids in extracts of both plants might have contributed to their antimalarial activities. Flavonoids were also present in the aqueous and methanol extracts of the leaves, stem and roots of *C. dependens* and *D. arborescens*. Flavonoids are compounds which occur widely in the plant kingdom and they have also been detected in *Artemisia* species. They are reported to have shown significant *in vitro* antimalarial activity against *P. falciparum* (Chanphen, Thebtaranonth, Wanaupathamkul & Yuthavong, 2018). Their presence in *C.dependens* and *D.arborescens* plants justify the antimalarial activities exhibited by these plants' extracts. They also have protective effects against platelet aggregation, allergies, free radical, microbes, ulcers, hepatoxins, viruses and tumour (Farquar, 1996; Okwu, 2004).

Cardiac glycosides were present in the various extracts from the two plants. This class of phytochemicals are referred to as 'natural drugs' because their actions help in treating heart related problems (Radford, 2006). Glycosides have blood pressure lowering effects (Marinkovic & Vitale, 2008).

Aqueous and methanolic extracts of the leaves, stem and roots of the two plants contain phenols. Phenol is a common phytochemical in plants. Phenolic extracts are the most naturally occurring natural products known for allelopathic activities (Singh, Batish & Kohli, 2013). Activities of phenols also include antioxidant, anti-inflammatory, and antimalarial activities (Khahkonen, Hopia, Vuorela, Rauha, Pihlaja & Kujala, 2009; Ovenden, Cobbe, Kissell, Birrell, Chavchich &

Edstein, 2011; Han, Shen & Lou, 2017). Steroids which were also identified in the plant extracts have been reported to have anti-inflammation effects (Savithramma & Linga, 2011). Saponins also have anti-hyper cholesterol and haemolytic effects (Sodipo, Akiniyi & Ogunbamosu, 2010).

Results obtained in this study are in agreement with Alghazeer and El-Satani (2012) who also reported the presence of alkaloids, saponins, tannins and terpenoids in screened medicinal plants. These secondary metabolites are major sources of food additives, pesticides, herbicides and pharmaceuticals (Ogidi, Omu & Ezeagba, 2019; Rainu & Mohan 2012).

Continous search for plants with high therapeutic values has continued to reveal information on medicinal properties of plants, and their uses in the management of various ailments. These therapeutic properties of plants have a link with the phytochemical components of these plants. Since plant resources are reported to be safer, affordable and more accessible than synthetic products, their continous usage is therefore recommended (Ikpeme, Udensi, Ekerette & Chukwurah, 2013; 2014; 2015; Ekaluo, Ikpeme, Ekerette & Chukwu, 2015). Therapeutic properties of plants are credited to the antioxidant compounds and phytochemicals present in these medicinal plants (Padmanabhan & Jangle, 2012). Antioxidant compounds have the ability to chelate and bind free radicals into removeable forms such that they can easily be excreted from the body. This property of antioxidant compounds are attributed to their redox properties which give them the ability to act as reducing agents, electron donors or metal chelation (Demiray, Pintado & Castro 2009; Premanath & Lakshmidevi, 2010). Antioxidants are known for their ability to scavenge free radicals, ameliorate free radical related diseases and conditions, such as oxidative stress. Therefore plants with antioxidant properties are of great value to traditional medicine. Free radicals are implicated in many disorders, such as cancer, rheumatoid arthritis and

neurodegenerative diseases. Extracts of the leaves, stem and roots of *D. arborescens* and *C. dependens* showed appreciable antioxidant activities. *In vitro* antioxidant activities of the extracts were compared to that of ascorbic acid (Vitamin C) which was used as the standard antioxidant.

Reduction of DPPH free radical works on the principle that the antioxidant reacts with the stable free radical DPPH, converting it to 1, 1- diphenyl-2-picryl hydrazine (Sreeyan & Rao, 1997). Addition of the various extracts to DPPH solution caused a rapid decrease in absorbance at 518 nm. This indicated that the extracts have good scavenging activity (Tables 4.6 and 4.7).

Aqueous and methanolic extracts of the leaves, stem and roots of *D. arborescens* and *C. dependens* showed DPPH scavenging activities in a concentration dependent-manner with DPPH scavenging activities increasing with increase in concentration of extracts. Highest activity, with IC_{50} of 1.19 ± 0.08 mg/ml was recorded for the aqueous extract of *D. arborescens* root. This result differed significantly ($p<0.05$) from the standard antioxidant (Ascorbic acid) whose IC_{50} was 0.58 ± 0.05 mg/ml. The least DPPH scavenging activity with an IC50 value of 1.66 ± 0.27 mg/ml, was recorded for methanolic extract of *D. arborescens* root. Secondary metabolites, which are usually natural antioxidants, could be therapeutic (Herrera & Barbas, 2011). In this study, the extracts showed effective scavenging activities against DPPH radical. Data obtained shows that the more the decolourization of DPPH, the more the reducing ability of the extracts (Pacher, Steffens, Hasko, Schindler & Kunos, 2017). This indicated that the extracts were capable of donating a hydrogen atom to the stable free radical (DPPH). Ability of the extracts to scavenge the DPPH radical increased in a concentration- dependent manner. DPPH radical scavenging abilities of the extracts followed a Sigmoid model (Table 4.6 and 4.7). High reduction of DPPH is related to the high scavenging activity of a particular sample (Molyneux, 2014). These scavenging abilities of

aqueous and methanolic extracts of the leaves, stem and roots of *D. arborescens* and *C. dependens* indicated that they possess good antioxidant activities which could be attributed to the synergistic activities of the phytochemicals present in these plants. These plants are rich in various phytochemical compounds as recorded in their quantitative screening. Results obtained were in agreement with Asadu, Abonyi, Anosike, Uzoegwu & Uroko, (2018), who reported that methanol extract of *Lantana camara* leaves scavenged DPPH radical in a concentration-dependent manner. These results were also in line with findings of Alisi, Asiwe, Ene, and Alisi, (2018) and Shruthi, Roshan, Timilsina, & Sunita, (2013). These plant extracts thus have components which are capable of scavenging free radicals through electron donation. As a result, they have the potential to prevent effects of free radicals in biological systems.

Nitric oxide (NO) is reported to be involved in many biological functions, such as neurotransmission, antimicrobial and antitumor activities, as well as vascular homeostasis (Ghafourifar & Cadenas, 2005). Despite the beneficial effects of nitric oxide, it contributes to oxidative damage. This is because NO can react with superoxide ion to form peroxynitrite anion which is a strong oxidant that can decompose to produce OH and NO_2 (Toykuni, 2019). The present study showed that aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* and *C. dependens* have potent nitric oxide scavenging activities. These extracts showed strong NO radical scavenging activities which reduced the nitrite ion concentration in the *in vitro* assay medium thereby exhibiting protective effect against oxidative damage. Nitric oxide scavenging activities were concentration-dependent, as increase in concentration of extracts increased nitric oxide scavenging activities of the extracts. Aqueous extract of *D. arborescens* root recorded the highest nitric oxide activity (IC_{50} = 0.72 ± 0.11 mg/ml). This activity differed significantly ($p<0.05$) from 0.65 ± 0.07 mg/ml recorded as the IC_{50} for the standard antioxidant

(Ascorbic acid). Ability of these extracts to scavenge nitric oxide generated *in vitro* by sodium nitroprusside followed a sigmoid model. Nitric oxide scavenging activities recorded in this study could be attributed to secondary metabolites, such as tannins, saponnins, flavonoids and carotenoids present in these plant extracts (Traber & Atkinson, 2007). Results obtained in this study agree with reports of Alisi and Onyeze, (2008) and Jagetia, Rosk & Babu (2014).

This study also showed that aqueous and methanol extracts of the leaves, stem and roots of *D.arborescens* and *C.dependens* scavenged hydrogen peroxide (H_2O_2) effectively, compared to Ascorbic acid (standard antioxidant). Hydrogen peroxide (H_2O_2) easily decomposes into oxygen and water which may produce hydroxyl (OH) radicals that can initiate lipid peroxidation causing damage to DNA. The highest H_2O_2 scavenging activity (IC_{50} =0.42 ± 0.00) was recorded for methanol extract of D.arborescens root. This activity, as well as activities of the other extracts, were significantly lower ($p<0.05$) than that of ascorbic acid. Ability of the extracts to scavenge H_2O_2 followed a sigmoid model while the standard antioxidant (ascorbic acid) followed logistic dose response model. Both aqueous and methanolic extracts scavenged H_2O_2, and this is attibuted to presence of tannins, phenols, saponins etc present in the extracts; these neutralize H_2O_2 to water. These phytochemicals were qualitatively and quantitatively present in extracts of the two plants.

Reducing power ability of extracts indicates presence reducing agents whose atoms have capacity to donate electrons or react with free radicals, and convert them into stable metabolites, terminating radical chain reactions (Ganu, Jadhav & Deshpande, 2010). Some studies have shown that reducing power of an extract indicates possible antioxidant properties due to presence of reductants (Chanda, Dave & Kaneria, 2011). Ekaluo *et al*. (2015) opined that the reductants present in the extracts promote reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). As concentration

of Fe^{2+} increases, absorbance value increases indicating the extract's electron donating ability as recorded in this study. Antioxidant properties of the extracts were confirmed by their ferric reducing antioxidant power (FRAP). Ferric reducing antioxidant power of the extracts followed logistic dose response and sigmoid a, b, c model, except methanol extract of *C.dependens* leaves which followed Weibull cumulative [WeibullCum a,b,c,d)] model. The plant extracts possession of ferric reducing antioxidant power indicated ability of the bioactive components present in the plant extracts to donate hydrogen. All the extracts showed good reducing power (Tables 4.12 and 4.13). Many antioxidants and pharcomacological agents used in treating diseases associated with oxidative stress have been reported to have high reducing power (Amin & Razieh, 2007). This equally agrees with Shiddhuraju, Mohan & Becker, (2012) who reported that ferric reducing power of most biologically active compounds are linked with antioxidant activity.

For total antioxidant capacity (TAC), the highest value (2352.94 ± 164.87 mg AA/g extract) was recorded for methanolic extract of *D. arborescens* root. Activities of these extracts suggest that they have antioxidant components which could be compared to those of ascorbic acid which was used as a reference standard in this study. Data obtained agree with the findings of Aderogba, Okoh & Idowu, (2005) who evaluated the antioxidant activity of the secondary metabolites from *Piliostigma reticulatum*. Antioxidant activities of the extracts can be attributed to their flavonoid content and agrees with the findings of Sunil (2014) who reported that flavonoids promote antioxidant activities, cellular health, normal tissue growth and body renewal. Results from this study also corroborates the findings of Alisi *et al*. (2011) who reported free radical scavenging and *in vitro* antioxidant effects of ethanol extract of *Chromolaena odorata* Linn.

Toxicity is an expression of the poisonous potential of any extract. It reveals the state of adverse effects which is caused by interaction between the toxicants and cells. For the evaluation and assessment of toxicity of medicinal plants, determination of acute oral toxicity (LD_{50}) is usually a preliminary step. Testing for toxicity is very important in the screening of new drugs or compounds prior to its use on humans. Toxicity testing determines potential hazards which may be produced by a test substance and characterizes the action of such toxicants. Toxicity tests are usually carried out on experimental animals (Cunny & Hodgson, 2009). Acute toxicity test (dose which can kill 50% of experimental animals) is a short term evaluation of potential hazards by a test substance (Monosson, 2013). Results obtained from acute toxicity studies pave way for dose determination in animal studies, aid in the determination of LD_{50} values which provide many indices of potential types of drug activity. It equally helps to arrive at an appropriate dose of a new drug (compound) to be used in prolonged studies as well as the basis for which other testing programs may be designed.

The experimental mice were orally treated with single dose each of 10 to 5000 mg/kg body weight of the extracts. Data obtained from the acute toxicity study indicated no sign of toxicity or mortality within 24 hours and subsequently for 7 days in both phases of the study for aqueous and methanol extracts of the leaves, stem and roots of *D. arborescens* plant. Similar findings were recorded for the aqueous and methanol extracts of leaves and roots of *C. dependens* plant. These extracts did not produce any physical signs of toxicity, such as paw licking, salivation, stretching, weakness or calmness. Their LD_{50} was thus estimated to be ≥5000 mg kg^{-1} because no mortality was recorded even at this high dose of 5000 mg kg^{-1} body wight of the extracts. Absence of mortality in the test animals following oral administration of these extracts at 5000 mg kg-1 body weight recorded indicated that the extracts were not toxic acutely (Salawu, Chindo, Tijani,

Obidike, Salawu & Akingbasa, 2009). These results are in consonance with the reports of Akuodor *et al*. (2012); Onwusonye, Uwakwe, Iwuanyanwu and Iheagwam, (2014), and Ihekwereme, Agbata, Agbata, Chukwueze and Agu, (2016) who reported no mortality in Swiss albino mice treated with 10 to 5000mg kg-1 body weights of leaf extract of *Aspilia africana* (Pers), methanol leaf extract of *Annona senegalensis* and hydroethanolic stem extract of *Baphia pubescens* respectively.

However, aqueous and methanol extracts of *C.dependens* stem recorded toxicity at 2900 and 5000 mg kg^{-1} body weight of the extract. Their LD_{50} was thus, estimated to be 3808 mg kg^{-1} body weight of the extract. This result agrees with the study of Khan, Amupitan, Oyewale and Ndukwe, (2015), who reported a median lethal dose of 3808 mg kg^{-1} body weight of methanolic leaf extract of *Nepata cateria* in albino mice.

There is presently much dependence on plant-derived products to manage diseases and infections. The two plants under study (*D. arborescens* and *C. dependens*) are some of such plants which are currently being exploited to mitigate against diseases and ailments. Various biochemical and haematological parameters investigated in this study serve as useful indices in the evaluation of toxicity of plant extract in animals (Yakubu *et al*., 2008). Evaluation of haematological parameters is not only used to determine the extent of harmful effects of extracts on the blood of an animal, it is also used to elucidate blood relating functions of a plant extract or its products (Yakubu, Akanji & Oladiji 2007). Analysis of blood parameters is very important in risk assessment because changes in the haematological indices predicts human toxicity when the data are translated from animal studies (Olson, Betton, Robinson, Thomas, Monro, Kolaja, Lilly, & Sanders, 2010).

Haematological parameters are equally very important for assay because the hematopoetic system is a major target for toxic compounds. These parameters are also used to monitor the pathological and physiological status of animals and humans (Olson *et al*., 2010). Blood is the major transportation channel for food and foreign bodies, therefore, its components, such as hemoglobin, platelets, RBCs, and WBCs, are usually exposed to higher doses of toxic compounds thereby exposing these parameters to danger. When there is any damage in these blood cells, the immune system becomes compromised. Results obtained show that most of the extracts significantly increased ($p < 0.05$) haematological parameters compared to the normal and standard control. Increased values of WBC indicate that increase in the number of WBC is a normal reaction of rats to foreign substances, and this alters their normal physiological processes (Adebayo, Abolaji, Opata & Adegbenro, 2010). This process is called leukocytosis. Leucocytosis as recorded in this study indicates that there was a stimulation of the immune system which protects the rats against infections. This may have been caused by secondary infections. Leucocytosis is directly proportional to the severity of the causative stress condition and may be attributed to an increase in leukocyte mobilization (Celik and Suzek, 2008).

Reports have shown that some chemicals or conventional drugs have adverse effects on various blood components. Hematotoxicity occurs when these blood parameters are elevated beyond their normal ranges (Dioka, Orisakwe, Afonne, Agbasi, Akumka, Okonkwo & Ilondube, 2002). Red blood cells are highly susceptible to lipid peroxidation because they have unsaturated membrane lipids. Lipid peroxidation occurs when free radicals react with membrane lipids. This is considered a crucial feature of cellular injury (Odukoya & Adeniji, 2012). Increase in packed cell volume, heamoglobin, white blood cells, red blood cells, mean corposcular heamoglobin, mean corposcular heamoglobin concentration, neutrophils, and lymphocytes in the experimental animals suggests

that extracts of the leaves, stem and roots of *D.arbrescens* and *C. dependens* may have haematopoietic properties which will promote erythropoiesis in animals. This can increase resistance to oxidative damage to the membranes of red blood cells. Findings in this study are in agreement with previous study by Nwinuka, Monanu & Nwiloh, (2008) who reported that *M. indica* stem bark extract had the ability to boost haematopoietic system of rats. Ogbe, Yahaya & Anaefu, (2010) also reported that *M. indica* stem bark extract possesses antianaemic properties; these could improve erythropoiesis and reduce oxidative damage to RBC membranes, thus preventing exposure of the erythrocytes to destruction by macrophages.

The significant increase in WBC counts in the experimental animals also suggests that the extracts may have immunological properties, which stimulated increased production of white blood cells, thus boosting the defence system of the animals. These results are consistent with the findings of Ukoha *et al*. (2017) who reported that aqueous dried leaf extract of *Catharanthus roseus* improved heamatological indices in male albino rats. Findings in this study also indicate that the aqueous and methanol extracts of the leaves, stem and roots of *D. arborescens* and *C. dependens* may not have had adverse effects on haematological parameters and are therefore nontoxic. It further justifies the use of these plants in traditional medicine.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) have been identified as indicators of hepatocellular injury, while Alkaline phosphatase (ALP) is a marker of cholestasis (Abu & Uchendu, 2010). Thus, results obtained show that aqueous and methanol extracts of the leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* plants did not significantly elevate the activities of liver function enzymes AST, ALT and ALP in the serum of test animals. This suggests that extracts of the leaves, stem, and roots of these two plants may not

induce hepatocellular injury in animals. Decrease in the level of these enzymes in the serum of test animals may be due to non- leakage of hepatocytes which normally occurs from peroxidative damage of their membranes leading to increased membrane permeability (Iniaghe, Malomo, Adebayo, 2009). The significant reductions recorded in the activities of these enzymes indicates that extracts of the different parts of the plants were not harmful to the liver. This agrees with the findings of Adebayo, Abolaji, Opata and Adegbenro, (2010) who reported that ethanolic leaf extract of *Chrysophyllum albidum* reduced levels of ALT, AST, ALP, total protein, albumin and other biochemical parameters.

Serum albumin and total protein are some of the markers of liver dysfunction, albumin transports bilirubin and other substances in blood (Ogbe, Adoga & Abu, 2012). Significant increase in serum total protein is an indication of tissue damage while a significant decrease in total protein of the liver contents suggests hepatic toxicity (Gatsing, Aliyu, Kuiate, Garba, Jaryum, Tedongma, Tchouanguep & Adoga, 2005). Although extracts used in this study might have slightly increased or decreased the levels of total protein and albumin in the experimental animals, the non significant difference in the levels of these biochemical parameters when compared with the normal and standard control suggests that extracts used in this study may not interfere significantly with the metabolism of these biochemical parameters. This also agrees with the findings report of Unuofin and Otunola, (2018) who studied acute and subacute toxicity of aqueous extract of the tuber of *Kedrostis africana* (L.) in Wistar rats. They reported that daily oral administration of the extract did not significantly change hematological or biochemical parameters at the highest dose of 600 mg/kg and no alteration in body weight, food and water intake was recorded.

Results equally agree with the findings of Uboh, Okon and Ekong, (2010) who evaluated effects of aqueous extract of *Psidium guajava* leaves on liver enzymes, histological intergrity and heamatological indices in rats. Their results revealed that the liver function enzymes, ALT, AST and ALP, as well as total protein and albumin in male and female rats were not significantly ($p>0.05$) affected by oral administration of the extract. Prasad & Venugopal (2016) similarly reported that extract of *Argemone mexicana* root did not record any evident clinical signs of toxicity and mortality during a 14-day test period, even with the highest dose (5000 mg/kg b.wt). Data obtained in this study also suggests that the aqueous and methanol extracts of leaves, stem and roots of the two plants did not have any negative impact, rather seemed to have a protective effect on heamatological and biochemical parameters.

Results of acute and sub-acute toxicity in this study equally agree with the findings of Idowu, Soniran, Ajana and Aworinde, (2009); Subramanion, Zuraini, Yeng, Yee, Lachimanan and Sreenivasan, (2011), and Roy, Ukil & Lyndem, (2016) in their studies. They reported no toxicity with *C. citrates*, *Cassia fistula*, and *Senna alata* extracts.

Africa is gifted with a myriad of herbal medicines. Indigenous people acquire this knowledge, preserve it and pass on to their generations. Aqueous and methanolic extracts of *C. dependens* and *D. arborescens* leaves, stem and roots showed anti-malarial activity against *P. berghei* infection inmale Swiss albino mice as recorded in the reduction of parasite count and decrease in percentage parasitemia. Results obtained from the *in vivo* anti malarial studies show that methanolic extracts of leaves, stem and roots of *C. dependens* and *D. arborescens* exhibited higher antimalarial activities on *P. berghei* infected mice than the aqueous extracts. Difference between the antimalarial activities of methanol extracts of the roots and leaves of *D. arborescens* as well as

methanol extracts of *C. dependens* leaves differed significantly ($p<0.05$) from their aqueous extracts. This indicates that the bioactive components were more soluble in methanol than the aqueous medium (Peace, Ekaete, Chinweizu & Ruth, 2011). This result agrees with the report of Ene *et al*. (2013). Methanol is less dense than water and might diffuse more in a medium than water. Another possibility is that the active components were more soluble in methanol than in water which gave methanol extracts the benefit of having more of the bioactive compounds. This may be responsible for the higher antimalarial activity exhibited by the methanol extracts compared with the aqueous extracts. This corroborates the report of Ezeokeke, Ene and Igwe (2015). Aqueous and methanolic extracts of the leaves, stem and roots of the two plants under study significantly reduced ($p<0.05$) parasite count as well as percentage parasitemia compared with the negative control. Among the extracts, methanol extracts of the roots and leaves of *D. arborescens* showed the highest antimalarial activity, indicating that this plant is endowed with antimalarial activity. This confirms report that plants in the *Rubiaceae* family possess antimalarial activity. Asangha (2017) in a study '*In vivo* antiplasmodial activities of *Nauclea latifolia*' and 'Hematological indices of *Plasmodium berghei* infected mice treated with ethanol extract and fractions of *Nauclea latifolia* roots' reported this. Although aqueous and methanol extracts of the roots of both plants exhibited more antimalarial activities than aqueous and methanol extracts of the leaves, the differences was not that significant ($p<0.05$). Activities of the aqueous and methanol extracts of the stem differed significantly ($p<0.05$) from those of the roots and leaves. Previous studies on mice had reported significant antimalarial activities in the roots of plants (Andrade-Neto, Brandao, Stehman, Oliveira & Krettli, 2013; Muthaura, Rukunga, Chhabra, Omar, Guantaiu & Hathirwa, 2017). Antimalarial activities of the aqueous and methanol extracts of the leaves of both plants were next to that of the roots. Leaves of medicinal plants are extensively used

in herbal remedies than other parts of the plants. Results in this study corroborates findings of Ajaiyeoba, Oladepo, Fawole, Bolaji, Akinboye & Ogundahunsi, (2003) and Idowu, Soniran, Ajana and Aworinde, (2010). Aqueous and methanol extracts of the stem of both plants exhibited the least antimalarial activities on *Plasmodium berghei* infected mice. These may be due to the fact that the stems of both plants are not woody. Earlier reports showed that most plants with significant antimalarial activities in their stem extracts are trees with stem bark. This agrees with the findings of Hilou, Nacoulma and Guiguemde, (2016) and Muthaura *et al*. (2017), who reported significant antimalarial activities in the stem bark extracts of *Amaranthus spinosus* and *Boerhaavia erecta* which increased with dosage and 84.85% chemosuppression in the stem bark water extract of *Warburgia stulhmanni* in mice respectively. Percentage parasitaemia in the negative control group on Day 3 after infection with *Plasmodium berghei* parasites was 63.0%; it gradually rose to 72.4% on Day 7 and 79.2% on Day 14. Parasitaemia in the negative control group was higher than in all the treatment groups, indicating that all the extracts had effect on the growth of *Plasmodium berghei* parasites in mice. This study also shows the antimalarial activities of the two plants under study (*C. dependens* and *D. arborescens*) in the solvents used. During post-treatment days, aqueous and methanol extracts of the leaves, stem and roots of both plants further reduced ($p<0.05$) parasitemia compared with the untreated control. Methanolic extract of the root of *D. arborescens* plant exhibited best post-treatment effect in mice. Survival rate of mice treated with the various extracts supported the prolonged effects of the antimalarial compounds in these two plants. Although aqueous and methanol extracts of the stem exhibited post-treatment antimalarial activities, they had the least weight gain and survival rate. This may be attributed to the type of antimalarial compound present in the stems, and their quantities. These results are in line with the report of Soniran, Idowu & Idowu, (2011), who evaluated *in vivo* antiplasmodial activities of

extracts *Morinda morindiodes* (Bak.) in the treatment of malaria. Findings are also in agreement with Enenebeaku, Ukwandu, Mgbemena, Nwigwe, Enenebeaku, Duru & Ogidi, (2021a).

When compared to the standard antimalarial drug (artesunate), aqueous and methanolic extracts of *C. dependens* and *D. arborescens* leaves, stem and roots did not achieve total eradication of the parasites rather they exhibited varying degerees of antimalarial activities compared to the standard antimalarial drug (artesunate). Standard antimalarial drugs are more effective, but they are associated with several problems in the management of malaria. These include development of resistance, production and circulation of fake antimalarial drugs. Moreso, these drugs are not readily available and they are sold at exhorbitant prices. Therefore use of these herbs and medicinal plants should be advocated (Mgbemena, Opara, Ukaoma, Ofodu, Njoku & Ogbuagu, 2010).

Body weight of the animals in all the infected and treated groups gradually increased when compared to the negative control. Efficacy of the plant extracts on *Plasmodium berghei* infected mice may be responsible. Decrease in percentage (%) body weight recorded in animals in the negative control group may be attributed to parasite feeding on the blood cells of the animals resulting in reduction of their body weight. This agrees with the reports of Ene *et al*. (2013).

Survival rate of animals in the treatment groups compared with the negative control group indicated that extracts used in this study were not toxic to the experimental animals at the administered doses. Recorded deaths would be attributed to the effects of the parasites. This agreed with the report of Adeyemo-Salami *et al*. (2014). The antimalarial activities of extracts from *C. dependens* and *D. arborescens* could be attributed to the alkaloids, flavonoids and

terpenoids contained in these plants Akuodor, Anyalewechi, Ikoro, Akpan, Megwas & Iwuanyanwu, (2010).

Results obtained for antimalarial activities of the extracts agree with findings of Babatunde *et al*. (2015) who reported significant chemosuppression of parasite growth in *Plasmodium berghei* infected mice with graded doses (100 to 800 mg/kg) of acetone extract of *T. orientalis.* It also agrees with Akuodor, Wande and Oyindamola, (2015) who reported that leaf extract of the plant *Pseudocedrala kotschyi* (100-400 mg/kg b.wt) showed significant dose-dependent activity against the *Plasmodium berghei* infected mice in suppressive and curative tests.

Results obtained here justify reports of Matur, Mathew and Ifeanyi (2009) and Alshawsh, Mothana, Al-Shamahy, Alsllami and Lindequist (2017) that plants containing numerous phytochemicals as their bioactive principle have antimalarial activities.

From the GC-FID analysis, various classes of phytochemicals were identified in eluates A and B from hexane fraction of methanolic extract of *D. arborescens* roots. Some of these phytoconstituents were present in both eluates but in different quantities. Epihedrin, humulone, epicatechin and resveratol were not present in eluate A but were present in eluate B while cohumulone, tannins and flavonones were present in eluate A but were not present in eluate B. These phytochemicals have a wide range of therapeutic activities in the human body.

Proanthocyanins form a unique class of high- molecular weight oligomeric and polymeric secondary metabolites which are usually present in fruits and other parts of the plant. They exhibit a wide range of biological activities in the human body; they include chemopreventive, vasorelaxing (Ortega, De-La-Hera, Carreto, Gomez- Serranillos, Naval, Villar, Prodanov & Vacas, 2008) antoxidative (Sanchez-Moreno, Cao, Ou & Prior 2013), anticancer/antiproliferative

(Schmidt, Howell, McEniry, Knight, Seigler, Erdman & Lila, 2014) effects. Anthocyanins are flavonoid compounds found in different parts of several plants. Reports show that they have antimalarial, anti-cancer and antioxidant effects (Seeram, Schutzki, Chandra & Nair, 2012). Tannins are polyphenols with several pharmacological activities, such as anti-inflammatory, antioxidant, antibacterial, antimalarial, anti-diarrheal, and antivirus (Oritz & Fragoso, 2005).

Flavanones have antimalarial properties (Murti & Mishra, 2014). Studies have shown that kaempferol has anti-microbial activity and antimalarial effects (Quarenghi, Tereschuk, Baigori & Abdala 2010; Singh, Pandey, Singh & Singh, 2018). Catechin is hemostatic in nature, cohumulone and humulone have a wide range of physiological effects which include anti-inflammatory, antioxidant, antiproliferative, antimalarial, anti-infective, antimicrobial, chemopreventive activities (Gerhauser, Alt, Heiss, Gamal-Elden, Klimo, Knauft & Newmann, 2002; 2005a; 2005b). Spartein Spartein is one of the quinoline alkaloids. Quinoline alkaloids are pharmacologically active compounds that have physiological effects, such as antimicrobial, antiinflammatory, and antimalarial (Kinghorn & Balandin 2014; Marella, Tanwar, Saha, Ali, Srivastava & Akhter, 2013). Sapogenins are steroid or triterpenoid glycosides, common in large number of plants and plant products. Their biological activities include antimicrobial and antimalarial activities (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007). Naringin is a flavanone glycoside; it is a very strong antioxidant (Jung, Kim, Lee, Lee, Kim, Park, Kim, Jeong & Chois, 2013). Epicatechin is very effective against free radical scavenging species (Goncalves, Bento, Fabio & Luis, 2017). Epihedrine is an alkaloid, it is used as a bronchodilator and decongestant. Resveratol has diverse biochemical and physiological effects which include anti-inflammatory, antioxidant and immunolatory activities (Kalantari & Das, 2010). Since most of these phytochemicals have

antimalarial activities, their synergistic effect may have been responsible for the antimalarial activities of these eluates.

Plants are important sources of potential useful bioactive compounds for the development of new therapeutic agents (Ogidi *et al*., 2019). The wavelength spectra recorded in the Fourier Transform Infra-Red (FTIR) spectroscopy of the two eluates from hexane soluble portion of methanol extract of *D.arborescens* roots served as a characteristic medium for elucidating the inherent functional group of organic compounds in the plant (Geethu, Suchitra, Kavitha, Aswathy & Dinesh, 2014). The infra-red spectra of the eluates showed different peaks signifying transitions between vibration levels of different molecule. FTIR spectra were used to identify the functional groups of the bioactive components in the eluates that were characterized based on the peak values in the region of IR radiation. As the eluates were passed through the FTIR, functional groups of the components were separated based on the peak ratio. Results obtained from FTIR analysis revealed presence of functional groups, such as alkanes (-CH2-), non conjugated alkenes (C=C), carboxylic acids (C=O), esters (OC-H), etc.

Gas chromatography- Mass spectrometer (GC-MS) is a vital tool in the discovery and identific tion of therapeutic chemical components in plants (Mokaya, Omosa, Ogunnah & Nyamato 2021). GC-MS was used to identify the bioactive compounds present in the two eluates.

Table 4.37: Biological activities of some compounds identified in GC-MS analysis of eluate A (E_A) and eluate B (E_B) from hexane fraction of methanol extract of *D.arborescens* roots

S/N	Name of compound	Biological activity
1.	1- Octadecene	Antioxidant, antibacterial, antimalarial (Mishra & Sree, 2007)
2.	n-Hexadecanoic acid	Anti-inflammatory, antioxidant, mosquito larvicide, nematicide (Rahuman, Gopalakrishan, Ghouse, Arumugan & Himalayan 2000; Aparna, Dileep, Mandal, Karthe, Sadasivan & Haridas, 2012)
3.	9,17 Octadecadienal, (Z)-	Antimicrobial (Rajeswari, Murugan & Mohan, 2013)
4.	6-Octadecenoic acid methyl ester (Z)-	Anti-inflammatory, antioxidant, anticancer (Geetha & Varalakshmi, 2001)
5.	Hexadecanoic acid, methyl ester	Antioxidant, antimicrobial, hypocholesterolemic, nematicide, pesticide, antiandrogenic, insecticide (Akpuaka, Ekwenchi, Dashak & Dilda, 2013)
6.	Heptadecanoic acid, 16 methyl-, methyl ester	Anticancer (Elaiyaraja & Chandramohan 2018)
7.	Bis (2-ethylhexyl) phthalate	Antimicrobial, antifungal (Habib & Karim, (2009), antitumor (Habib & Karim, 2012)
8.	5- Octadecene	Antioxidant, antibacterial, antifungal (Narajani, Mangammi, Muvva, Poda & Munaganti 2016)
9.	Isobutyl tetradecyl ester	Antibacterial (Singh, Dar & Sharma, 2012)
10.	10- Octadecenoic acid, methyl ester	Antioxidant, antibacterial, antifungal, decreases blood cholesterol (Asghar & Choudhary, 2011)
11.	Cyclopropane octanoic acid, 2-hexyl-, methyl ester	Antimicrobial (Mishra & Sree, 2009)
12.	Methyl tetradecanoate	Lowers blood cholesterol (Micha & Mozaffarian, 2010), antimicrobial (Singh & Chaturvedi, 2019)
13.	2-Methyl-2,3- divinyloxirane	Adhesive (Ramalakshmi, Muthuchalian & Chudry, 2011)

Emergence of resistant strains of *Plasmodium* species to conventional drugs has motivated the search for new antimalarials with improved modes of action (Kell, 1991). One of the approaches to this problem is identification of enzymes that play vital roles in the life of the parasites and have notably different properties to enzymes which catalyse similar reactions in the human hosts (Sessions, Dewar, Victoria, Clarke & Holbrook, 2017). Such differences are exploited in the design of inhibitors to specific parasite proteins with the aim of developing pharmaceutical products that will target the disease. In the fight against malaria, lactate dehydrogenase from plasmodium species has been a major target due to the essential role this enzyme plays in the anaerobic lifestyle of *Plasmodium* species. Therefore any compound that inhibits the enzyme will also kill the *Plasmodium* (Royer, Deck, Campos, Hunsaker & Vander-Jagt, 2006).

The antimalaial activity demonstrated by hexane fraction of methanol extract of *D. arborescens* root extract was further validated by the detection of some bioactive compounds through GC-MS and molecular docking analyses. The compounds demonstrated varying levels of binding affinities for the two protein targets. Molecular interactions with critical proteins of the antimalarial pathways could modulate their activities and subsequently regulate malaria treatment (Enenebeaku, Duru, Mgbemena, Ukwandu, Nwigwe, Enenebeaku & Okotcha, 2021b).

All the chemical compounds obtained from the results of GC-MS analyses were subjected to molecular docking with two antimalarial protein targets. These molecular targets include *Plasmodium berghei* lactate dehydrogenase and plasmepsin II. Lactate dehydrogenase in *Plasmodium* species is an enzyme that catalyses synthesis of lactate from pyruvate (an important step in the fermentation process). *Plasmodium* species rely extensively on homolactic fermentation for energy production, therefore inhibiting this enezyme will disrupt synthesis of

lactate from pyruvate and energy production in the parasites will be altered (Winter, Cameron, Tranter, Sessions & Brady, 2013). This enzyme from *Plasmodium falciparum* and *Plasmodium berghei* is considered a potential molecular target for antimalarial drugs (Penna-Coutinho, Cortopassi, Oliveira, Franca & Krettli, 2011).

Plasmepsin II is a haemoglobin degrading protein in *Plasmodium* species. It is also a potential drug target for antimalarial drugs (Gutali, Narula, Vishnu, Katyal, Negi, Ajaz, Narula, Chauhan, Kant & Lumb, 2015). Degradation of hemoglobin is an important step in the development of malarial parasites. Degradation of hemoglobin by malarial parasites is catalysed by enzymes known as plasmepsins (PMs) (Francis, Sullivan & Goldberg, 2014). Plasmepsin II is an effective therapeutic target for the treatment of malaria. This enzyme degrades hemoglobin by proteolytic cleavage.

The binding energy of Bis(2-ethylhexyl) phthalate (-6.1 Kcal/mol and -6.6 Kcal/mol) and Bis(3-methylbutan-2-yl) phthalate (-6.2 Kcal/mol and -6.0 Kcal/mol) obtained for the two proteins were found to be close to that of the standard ligand or compound (artesunate) whose binding energy for the two proteins were -8.1 Kcal/mol and -7.5 Kcal/mol for lactate dehydrogense and plasmepsin II respectively. These compounds interacted with the amino acids at the binding pockets of the standard drug active sites using different types of chemical bonds. This indicates a competitive inhibition with lactate dehydrogenase and plasmepsin II in *Plasmodium berghei*, thus explaining the antimalarial activity exhibited by hexane fraction of methanol extract of *D.arborescens* root. This result agrees with the findings of Penna-Coutinho *et al*., (2011) who studied antimalarial activity of potential inhibitors of *Plasmodium falciparum* lactate dehydrogenase enzyme using docking studies selection. Data obtained is also in agreement with the findings of Gulati *et al*. (2015) who studied plasmepsin II as a potential drug target for resistant

malaria. The protein-ligand interaction was made possible by the presence of chemical bonds, such as Van der Waals's forces, conventional hydrogen bond, carbon hydrogen bond, Alkyl-alkyl bonds etc which held the compounds in the protein pockets.

Absorption, distribution, metabolism, elimination and toxicity (ADMET) properties of chemical compounds are important determinants in selecting a drug molecule (Cheng, Li, Zhou, Shen, Wu, Liu & Tang 2012; Duru, Duru, Ikpa, Enenebeaku, Obiagwu, Igbomezie & Nnabuchi, 2021).

The Lipinski's rule of five, determined by a complex balance of various molecular and structural properties, such as human intestinal arbsorption, water solubility, carcinogenicity, hepatotoxicity, Plasma protein binding etc which influence the behavior of a drug molecule in a living organism, were used to suggest the drug likeliness of the compounds. A good drug candidate should not violate more than one of the rules (Lipinski, 2016). ADMET properties of Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate suggested that these compounds are not harmful compared with the standard drug (artesunate). This justifies earlier results obtained for methanol extract of *D. arborescens* roots for acute and sub-acute toxicity studies in this study.

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study had shown that the crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens* contain various phytochemicals which were active against *Plasmodium berghei* and have high potentials as antimalarial agents justifying their use in traditional medicine especially in the treatment of malaria. Findings from this research equally reveal that these plants could be potential sources of natural antioxidants, preventing and/or slowing degenerative diseases. The extracts did not exhibit any chronic toxicity on experimental animals, suggesting no harmful effects following their use. Chromatographic studies of the methanolic extract of *D. arborescens* root showed that the eluates (fractions) possess significant antimalarial activity *in vivo*; hexane fraction of methanolic extract of *D. arborescens* root showed the highest activity. Studies on this fraction led to the isolation of two eluates **A** and **B** (E_A and E_B) which contain phytochemicals known for various therapeutic activities. These findings thus, lend credence to the ethnomedicinal claim for the use of these plants in the treatment of malaria. FTIR analysis revealed presence of functional groups such as; alkanes (-CH2-), non conjugated alkenes (C=C), esters (OC-H), etc. These were confirmed by the various compounds obtained by GC-MS analysis. Furthermore, bioactive compounds of the hexane fraction of methanolic extract of *D. arborescens* root in eluates A and B showed various levels of binding affinities and molecular interactions with two antimalarial protein targets, lactate dehydrogenase (1OC4) and Plasmepsin II (1SME). Binding affinities (docking scores) of Bis(2-ethylhexyl) phthalate and Bis(3-methylbutan-2-yl) phthalate were close to that of the standard antimalarial drug (artesunate) explaining the antimalarial property exhibited by this extract. The protein-ligand

interaction was made possible by the presence of chemical bonds, such as van der Waals' forces, conventional hydrogen bond, carbon hydrogen bond, and Alkyl-alkyl bonds which held the compounds in the protein pockets.

ADMET properties of the two compounds with best docking scores equally suggest no harmful or toxic effects compared with the standard drug. Antimalarial and antioxidant activities of *D. arborescens* could therefore be attributed to presence of these bioactive compounds. These findings therefore validate antimalarial potentials of the plant.

5.2 Recommendations

Since these plants are sourced locally, their continuous use in the treatment of malaria and generalized conditions caused by free radicals in the body is recommended.

Given that methanolic extract of *D.arborescens* root contains antimalarial compounds (Bis-2-ethylhexyl phthalate and Bis -3-methylbutan-2-yl phthalate), they can be synthesized as antimalarial therapies which can be included in some antimalarial formulations.

Chromatographic and bioassay guided isolation on the leaves of *D.arborescens* as well as leaves and roots of *Chasmanthera dependens* are recommended in order to establish their possession of similar compounds since they are also used in the treatment of malaria.

Studies on these plant parts using other concentrations of the extracts are also recommended. Scientific validation of these medicinal plants may eventually contribute to the integration of ethno-medicine into mainstream malaria management.

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APPENDICES

Appendix I: Result of packed cell volume and haemoglobin before and after treatment with aqueous and methanolic extracts of the leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens*

Treatments	**Haematological indices**			
(mg / kg)	**Before Treat.**	**After Treat.**	**Before Treat.**	**After Treat**
	PCV (%)	**PCV (%)**	**Hb (g/dl)**	**Hb (g/dl)**
NC	$31.3^{a} \pm 3.74$	$42.0^{a} \pm 3.81$	$14.0^{a} \pm 1.43$	$16.8^{a} \pm 1.51$
STD.C	$34.0^{a} \pm 3.40$	$42.0^{a} \pm 3.64$	$10.9^{b} \pm 1.23$	$16.0^{a} \pm 1.43$
DARM	$33.3^{a} \pm 3.55$	$42.3^{a} \pm 3.61$	$10.6^{b} \pm 1 .21$	$15.4^{a} \pm 1.34$
DALM	$33.0^{a} \pm 3.51$	$37.3^{b} \pm 3.43$	$10.3^{b} \pm 1.19$	$15.4^{a} \pm 1.34$
DASM	$28.9^{b} \pm 3.51$	$33.7^{c} \pm 3.50$	$10.2^{b} \pm 1.18$	$12.8^{b} \pm 1.18$
DARA	$31.3^{a} \pm 3.50$	$38.8^{b} \pm 3.54$	$10.4^{b} \pm 1.20$	$14.1^{a} \pm 1.31$
DALA	$32.0^{a} \pm 3.57$	$36.7^{b} \pm 3.50$	$11.0^{b} \pm 1.29$	$14.0^{a} \pm 1.30$
DASA	$31.4^{a} \pm 3.59$	$35.8^{b} \pm 3.44$	$10.5^{b} \pm 1.21$	$11.2^{b} \pm 1.14$
CDRM	$30.1^{ab} \pm 3.55$	$37.12^{b} \pm 3.48$	$10.8^{b} \pm 1.22$	$14.6^{a} \pm 1.35$
CDLM	$32.1^{a} \pm 3.35$	$36.5^{b} \pm 3.49$	$11.3^{b} \pm 1.34$	$14.3^{a} \pm 1.32$
CDSM	$31.3^{a} \pm 3.51$	$35.5^{b} \pm 3.31$	$11.2^{b} \pm 1.32$	$14.5^{a} \pm 1.13$
CDRA	$32.2^{a} \pm 3.36$	$37.5^{b}. \pm 3.45$	$11.2^{b} \pm 1.32$	$14.3^{a} \pm 1.11$
CDLA	$31.3^{a} \pm 3.56$	$36.5^{b} \pm 3.49$	$10.3^{b} \pm 1.19$	$13.8^{a} \pm 1.41$
CDSA	$30.3^{ab} \pm 3.56$	$34.8^{bc} \pm 3.29$	$10.5^{b} \pm 1.21$	$13.8^{a} \pm 1.41$

Appendix II: Result of white blood cells and red blood cells before and after treatment with aqueous and methanolic extracts of the leaves, stem and roots of *Chasmanthera dependens* and *Dictyandra arborescens*

Treatments	**Haematological indices**			
(mg / kg)	**Before Treat.**	**After Treat.**	**Before Treat.**	**After Treat.**
	WBC ($\times 10^{9}$/l)	**WBC ($\times 10^{9}$/l)**	**RBC ($\times 10^{12}$/l)**	**RBC ($\times 10^{12}$/l)**
NC	6.4^{b} ± 0.74	10.9^{ab} ± 0.47	6.8^{a} ± 0.33	8.1^{a}±0.31
STD.C	8.3^{a} ±0 .40	11.1^{b}± 0.80	5.3^{b}±0.30	8.3^{a}±0.32
DARM	8.7^{a} ± 0.46	12.1^{a} ± 0.91	5.1^{b} ±0 .28	8.3^{a}±0.32
DALM	9.5^{a} ± 0.84	12.2^{a} ± 0.92	4.6^{b} ± 0.23	8.1^{a}±0.31
DASM	8.4^{a} ± 0.41	10.9^{ab} ± 0.47	4.9^{b} ± 0.25	7.3^{b}±0.30
DARA	9.1^{a} ± 0.80	11.2^{a} ± 0.83	4.5^{b} ± 0.20	7.2^{b}±0.29
DALA	9.2^{a} ± 0.83	11.4^{a} ± 0.84	5.1^{b} ± 0.28	7.2^{b}±0.29
DASA	9.1^{a}± 0.59	11.1^{a} ± 0.81	5.2^{b} ± 0.29	8.9^{a}±0.34
CDRM	8.9^{a} ± 0.47	11.0^{a} ± 0.80	4.9^{b} ± 0.25	7.7^{a}±0.35
CDLM	9.4^{a} ± 0.84	11.3^{a} ± 0.84	4.7^{b} ± 0.24	6.9^{b}±0.25
CDSM	8.7^{a} ± 0.56	11.4^{a} ± 0.84	4.6^{b} ± 0.23	6.9^{b}±0.25
CDRA	9.1^{a}± 0.80	11.9^{a}. ± 0.88	4.4^{b} ± 0.21	6.9^{b}±0.25
CDLA	8.9^{a} ± 0.47	11.3^{a} ± 0.84	5.1^{b} ± 0.28	7.6^{b}±0.30
CDSA	8.6^{a} ± 0.46	11.0^{a} ± 0.81	4.7^{b} ± 0.23	7.8^{ab}±0.31