IN VITRO ANTIMICROBIAL AND *IN VIVO* ANTIMALARIAL EVALUATION OF LATEX AND COMPOUNDS ISOLATED FROM THE LEAVES OF *ALOE SINANA* Reynolds.

Genet Minale

A Thesis Submitted to the

Department of Pharmaceutical and Pharmacognosy

Presented in Partial fulfillment of the requirements for the degree of Master of Science in Pharmacognosy

Addis Ababa University

Addis Ababa, Ethiopia

April 2013

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Genet Minale, entitled: *"In vitro antimicrobial and in vivo antimalarial evaluation of latex and compounds isolated from the leaves of Aloe sinana."* and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Pharmacognosy) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External examiner_____ Signature_____ Date_____

Internal examiner_____ Signature_____ Date_____

Dr. Kalaeb Asres (Advisor) Signature_____ Date_____

Dr. Daniel Bisrat (Advisor) Signature_____ Date_____

Chair of Department or Graduate Program Coordinator

ABSTRACT

Aloe sinana Reynolds is endemic to Ethiopia where its leaf exudate is traditionally used in Debre Sina and in other central highlands of the country for the treatment of various illnesses, including malaria, wound and snake bite. Phytochemical investigation of the exudate led to the isolation of three anthrones identified as microdontin, aloin and aloinoside by means of spectroscopic techniques including ^{1}H and ^{13}C-NMR. The latex and isolated compounds were assessed for their antimicrobial activities against 20 bacterial and 4 fungal strains using disk diffusion method, and their antimalarial activities were evaluated by a 4-day suppressive test against mice infected with *Plasmodium berghei*.

When administered up to a dose of 5,000 mg/kg, neither the latex nor the isolated compounds were found to be acutely toxic to Swiss albino mice. Both the latex and isolated compounds showed potent antibacterial activity, whilst their effect against the tested fungal strains was rather moderate. In addition the latex and all the isolated compounds showed significant chemosuppression in mice infected with *P. berghei*. At a dose of 400 mg/kg, the latex suppressed parasite growth by 68.2% compared to the negative control group and improved survival time considerably. Among the isolated compounds, aloinoside showed the most potent antiplasmodial activity inhibiting parasite growth by 85.2% at a dose of 100 mg/kg.

From the results obtained in the present study, it can be concluded that the leaf exudate of *A. sinana* and its isolated compounds could serve as potential candidates for the treatment of microbial infections and malaria supporting the traditional uses of the plant.

Key words: *Aloe sinana*; Aloaceaae; antimalarial; antibacterial; anthraquinones; microdontin; aloin; aloinoside.

Acknowledgements

First I would like to thank the highest merciful GOD and St. Mary for giving me the strength and the calm throughout my study.

I wish to express my deepest appreciation to my advisors Dr. Kaleab Asres and Dr. Daniel Bisrat, for their invaluable comments and excellent supervision. I also extend my strong appreciation to Professor Sebsebe Demissew for identification of the plant material.

My appreciations are also extended to members of the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University for their technical and moral support when conducting the thesis work.

My deep appreciations are also forwarded to Ato Bikela, Ato Kemal, Ato H/Meskel, and Ato Getachew, for their assistance in Pharmacognosy laboratory; W/ro Amelework Eyado for her assistance during antimalarial test, Ato Mikias Tadesse, the animal attendant also deserves my appreciation.

My hearty thanks go to all my lovely family for their encouragement, appreciation and support towards my academic career. My sincere thanks also go to Heran, Meron, Yishamu, Abebu, Mebruka, Anwar, Tekleab, G/egzher, Mistere, Kidist, Bogale, Negasie, Kibenesh, Biruktawit, Fetene and to all my friends for their prayer and support.

Table of Contents

Acknowledgements ii

List of Abbreviations vii

List of Tables viii

List of Figures x

1. INTRODUCTION 1

1.1. Epidemiology of Malaria 1

1.2. The Malaria Life Cycle 2

1.3. Malaria Prevention, Control and Treatment 4

1.4. Medicinal Plants for Malaria Treatment 6

1.5. Ethiopian Medicinal Plants Used for Malaria Treatment 6

1.6. The Genus Aloe 7

1.6.1. Ethnobotany of *Aloe* 8

1.6.2. Pharmacological Activity 9

1.6.2.1. Antimicrobial Activity 9

1.6.2.2. Antioxidant Activity 9

1.6.2.3. Antiinflammatory Activity 10

1.6.2.4. Antimalarial Activity 10

1.6 3. Phytochemistry 11

1.6.4. *Aloe sinana* Reynolds 11

1.7. Statement of the Problem 13

2. OBJECTIVE OF THE STUDY 14

2.1. General Objective 14

2.2. Specific Objectives 14
3. MATERIALS AND METHODS 15
3.1. Materials 15
3.1.1. Plant Material 15
3.1.2. Chemical, Reagent and Drugs 15
3.1.3. Instruments 15
3.1.4. Experimental Animals 16
3.1.5. *Plasmodium berghei* 16
3.1.6. Microorganisms 16
3.2. Methods 17
3.2.1. Preparation of Plant Material 17
3.2.2. Chromatographic Techniques 17
3.2.2.1. Preparative Thin Layer Chromatography (PTLC) 17
3.2.2.2. Solvent System 17
3.2.2.3. Visualization 17
3.2.3. Spectroscopic Techniques 18
3.2.3.1. NMR, IR, UV and MS 18
3.2.4. Acid Hydrolysis 18
3.2.5. Acute Oral Toxicity Test 18
3.2.6. Determination of Antimicrobial Activities 19
3.2.5.1. Antibacterial Assay 19
3.2.5.2. Antifungal Assay 20
3.2.5.3. Minimum Inhibitory Concentrations (MIC) 20

3.2.7. Antimalarial Activity Test 21
3.2.7.1. Antimalarial Activity Test of the Latex and Isolated Compounds 21
3.2.8. Data Analysis 22
4. RESULTS AND DISCUSSION 23
4.1 Leaf Latex of A. sinana 23
4.1.1 Acute Toxicity 23
4.1.2. Antimicrobial Activity 23
4.1.2.1. Antibacterial Assay 23
4.1.2.2. Minimum Inhibitory Concentration (MIC) 25
4.1.2.3. Antifungal Activity 26
4.1.3. Antimalarial Activity of Leaf Latex of *A. sinana* 27
4.2. Compounds Isolated from the Latex of *A. sinana* 30
4.2.1. Isolation and Structural Elucidation 30
4.2.1.1. AS_1 30
4.2.1.2. AS_2 32
4.2.1.3. AS_3 34
4.2.2. Acute Toxicity of Isolated Compounds 37
4.2.3. Antimicrobial Activity 38
4.2.3.1. Antibacterial Assay 38
4.2.3.2. Minimum Inhibitory Concentration (MIC) 38
4.2.3.3. Antifungal Activity 42
4.2.4. Antimalarial Activity 44
4.2.4.1. Microdontin 44

4.2.4.2. Aloin 46

4.2.4.3. Aloinoside 48

5. CONCLUSION 52

6. RECOMMONDATION 53

Reference 54

Appendix ..... **Error! Bookmark not defined.**

List of Abbreviations

^{13}C NMR:	Carbon thirteen Nuclear Magnetic Resonance
^{1}H NMR:	Proton Nuclear Magnetic Resonance
ACT:	Artemisinin Combined Treatment
AIDS:	Acquired Immune Deficiency Syndrome
AL:	Artemether- lumefantrine
ANOVA:	One Way Analysis of Variance
DEPT:	Distortional Enhancement Polarization Transfer
DPPH:	2, 2-diphenyl-1-picrylhydrazyl
FRAP:	Ferric Reducing Antioxidant power
GDP:	Gross Domestic Product
HPLC:	High Performance Liquid Chromatography
IR:	Infra Red
IRS:	Indoor residual spraying
LLINS:	Long-lastig insecticide-treated mosquito nets
MIC:	Minimum Inhibitory Concentration
nr:	Not resolved
OECD:	Organisation for Economic Co-operation and Development
ORAC:	Oxygen Radical absorbance Capacity
RBCs:	Red Blood Cells
SP:	Sulfadoxin/ Pyrimitamine
SPSS:	Statistical Package for Social Science
TLC:	Tin Layer Chromatography
UV:	Ultra Violet
WHO:	World Health Organization

List of Tables

Table 1: Diameter of zone of inhibition and minimum inhibitory concentration (MIC) of the latex of *Aloe sinana* in comparison with that of ciprofloxacin. 24

Table 2: Antifungal activity of the leaf latex of *Aloe sinana.* 26

Table 3: Antimalarial activity of the latex of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei*. 27

Table 4: Body weight of *Plasmodium berghei* infected mice after administration of the latex of *Aloe sinana.* 29

Table 5: ^{1}H and ^{13}C NMR spectral data of $AS_{1.}$ 33

Table 6: ^{1}H and ^{13} C NMR spectral data of $AS_{2.}$ 35

Table 7: ^{1}H and ^{13} C NMR spectral data of $AS_{3.}$ 37

Table 8: Diameters of zone of inhibition of compounds isolated from leaf latex of *Aloe sinana* against some bacterial strains. 39

Table 9: Minimum inhibitory concentrations (MICs) of compounds isolated from the leaf latex of *Aloe sinana* against some bacterial strains. 41

Table 10: Diameters of zone of inhibition of the compounds isolated from the leaf latex of *Aloe sinana* at a concentration of 2000 µg/mL against some fungal pathogens. 43

Table 11: Minimum inhibitory concentration (MIC) values of compounds isolated from the leaf latex of *Aloe sinana* against some fungal pathogens..... 44

Table 12: Antimalarial activities of microdontin isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei*..... 45

Table 13: Body weight of *Plasmodium berghei* infected mice after administration of microdontin..... 46

Table 14: Antimalarial activity of aloin isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei*..... 47

Table 15: Body weight of *Plasmodium berghei* infected mice after administration of aloin..... 48

Table 16: Antimalarial activity of aloinoside isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei*..... 49

Table 17: Body weight of *Plasmodium berghei* infected mice after administration of aloinoside..... 50

List of Figures

Figure1: Life cycle of *Plasmodium* parasite 3

Figure2: The distribution of the genus *Aloe* is shown in orange 8

Figure3: Morphological view of *Aloe sinana*. 12

Figure4: TLC spots of the three compounds isolated from the leaf latex of *Aloe sinana* when viewed in day light, and under UV light of 254 nm and 366 nm. 30

Figure5: The structure of AS_1 (Microdontin)..... 32

Figure6: The structure of AS_2 (Aloin) 34

Figure7: The structure of AS_3 (Aloinoside). 37

1. INTRODUCTION

1.1. Epidemiology of Malaria

Malaria is a protozoal disease caused by parasites of the genus *Plasmodium* and is transmitted by female *Anopheline* mosquito (Karla *et al*., 2006). It is caused by four species of the genus *Plasmodium* namely: *P. falciparum, P. vivax, P. ovale,* and *P. malariae* (Francischetti *et al*., 2008). Apart from these, there is the simian parasite *P. knowlesi* that occasionally can infect humans, particularly in Malaysia (Lundqvist, 2009). The four species differ in microscopic appearance, clinical features, geographical distribution and the potential for development of resistance to antimalarial drugs. *P. falciparum* causes the most severe disease and is responsible for most malaria related deaths (Klotsas and Lever, 2007). It is by far the most common type of malaria parasite in Africa, and is also endemic in parts of Asia and South America. In Ethiopia, *P. falciparum* and *P. vivax* are the two dominant malaria species, accounting for 60% and 40% of infections, respectively (DFID, 2011; Mesfin *et al*., 2011).

Human malaria is the most important vector-borne disease and remains a major global health problem (Crawley *et al*., 2004). According to the estimates of the World Malaria Report (WHO, 2011), there were an estimated 216 million cases of malaria worldwide in 2010. The vast majority of cases (81%) were in the African Region followed by South-East Asia (13%) and Eastern Mediterranean Regions (5%). In the same year, there were an estimated 655,000 malaria deaths worldwide, of which 91% of deaths were in Africa. About 86% of deaths globally were in children under 5 years of age (WHO, 2011). Beyond the human toll, malaria has significant economic impacts in endemic countries, costs Africa more than US$ 10-12 billion in lost GDP

growth and it may have slowed down economic growth by as much as 1.3% per year (Okyere, 2011).

Ethiopia is also one of the most malaria-epidemic prone countries in Africa (UNICEF, 2010). According to World Malaria Report (WHO, 2011), there were an estimated 4,068,764 malaria cases and 1,581 deaths in Ethiopia in the year 2010. It was also the leading cause of outpatient visits and health facility admissions (ENMIS, 2011) and is second only to respiratory tract infections as a cause of death in children (Shargie *et al.*, 2008). Malaria is prevalent in over 75% of landmass; where areas of disease are primarily associated with altitude and rainfall. The peak of malaria illness incidence usually follows the main peak rainfall season (June to September) each year (ENMIS, 2011).

1.2. The Malaria Life Cycle

The human malaria parasite has a complex life cycle that requires both a human host and an insect host (Adorini *et al*., 2002). As shown in Figure 1, *Anopheline* mosquito inoculates plasmodium sporozoites (infectious form of the parasites) to initiate human infection. Circulating sporozoites rapidly invade liver cells, and exoerythrocytic stage tissue schizonts mature in the liver. Merozoites are subsequently released from the liver and invade erythrocytes (Katzung, 2006). Within the red blood cells, most merozoites go through another round of asexual reproduction, again forming schizonts filled with yet more merozoites. When the schizont matures, the cell ruptures and merozoites burst out and invade other RBCs, and the infection continues its cycle (NIAD, 2007). With rupture of the erythrocyte, the parasite's waste and cell debris is released into the blood stream, causing some of the clinical symptoms of malaria such as fever, chill, headache, abdominal and back pain, nausea, diarrhea, and

sometimes vomiting (Schlitzer, 2008). Severe disease can include delirium, metabolic acidosis, cerebral malaria and multi-organ system failure, coma and death (Fidock *et al*., 2004).

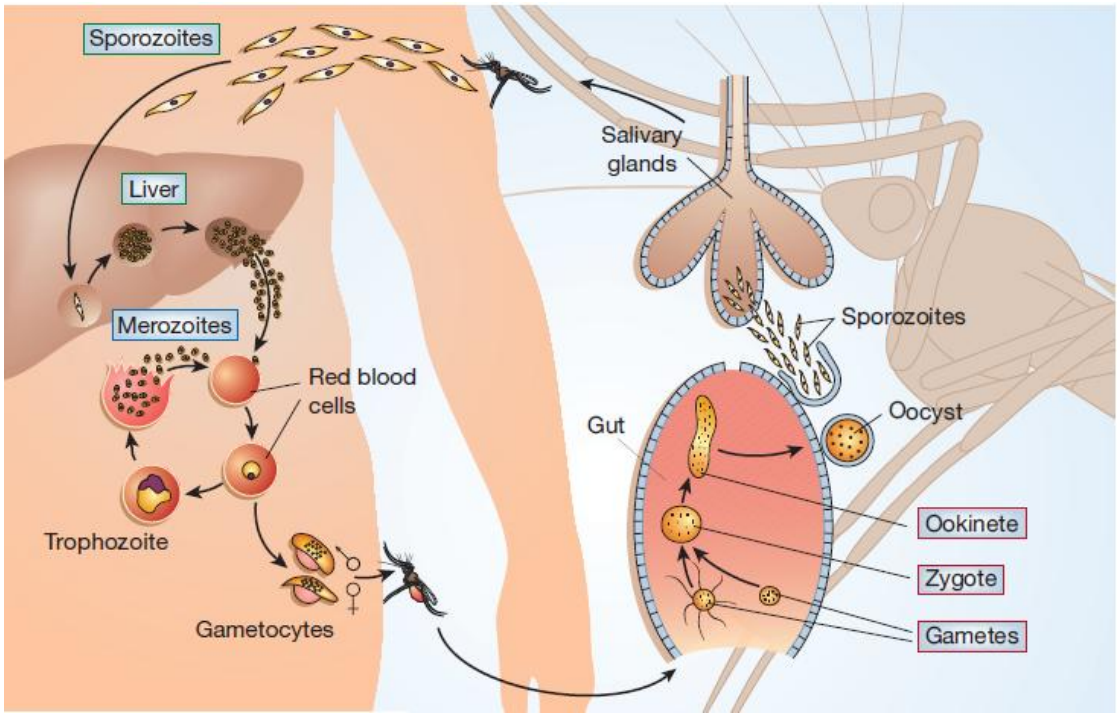


Figure 1: Life cycle of *Plasmodium* parasite (Lundqvist, 2009).

Once the tissue schizonts burst in *P. falciparum* and *P. malariae* infections, no forms of the parasite remain in the liver. However, in *P. vivax* and *P. ovale* infections, tissue parasites (hypnozoites) persist that can produce relapses of erythrocytic infection months to years after the primary attack (Shapiro and Glodberg, 2006).

Some merozoites differentiate into male or female gametocytes, which can be ingested by an *Anopheline* mosquito (Ongkana, 2003). In the mosquito's stomach, the gametocytes develop into male or female gametes. Fertilization occurs within the mosquito midgut, forming ookinete. These penetrate the midgut wall of mosquitoes to become oocyst. When the oocyst matures, it ruptures and releases sporozoites and they migrate to the mosquito's salivary glands waiting for injection to the human host through the mosquito bite during the next blood meal (Ongkana, 2003).

1.3. Malaria Prevention, Control and Treatment

Despite over a century of effort and early optimism, a malaria - free world remain as much distant vision as ever (Elufioye and Agbedahunsi, 2004). Many malaria control strategies exist, but none are appropriate and affordable in all contexts (Bloland, 2001). Some of the factors that contributed to this worst picture of malaria are high cost control programs, emergence of new insecticide resistant strains of the vector, creation of new mosquito breeding sites, the problem of drug resistance (*P. falciparum*) to almost all currently available antimalarial drugs, lack of organized health infrastructures and the migration behavior of people that increase the incidence and spread of malaria (Talisuna *et al*., 2004). Prevention and control of malaria include vector control with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) which reduce human-vector contact and the lifespan of female mosquitoes (WHO, 2011). Personal protective measures such as avoiding contact with mosquitoes during dust-to-down hours and wear clothes that cover most of the body are also critical for disease prevention (NEHC, 2000).

Appropriate treatment of sick individual with effective antimalarial drugs is accepted as principal strategies for malaria control worldwide (Kasa *et al*., 2005). Several classes of antimalarial drugs are available. Drugs that eliminate developing or dormant liver forms are called tissue schizonticides; those that act on erythrocytic parasites are blood schizonticides; and those that kill sexual stages and prevent transmission to mosquitoes are gametocides. No one available agent can eliminate both hepatic and erythrocytic stages. Few available agents are causal prophylactic drugs, i.e., capable of preventing erythrocytic infection. However, all effective chemoprophylactic agents kill erythrocytic parasites before they increase sufficiently in number to cause clinical disease (Katzung, 2006).

In the few areas where chloroquine-sensitive strains of *P. falciparum* are found, chloroquine is used for prophylaxis (Ongkana, 2003). It is also the prophylactic agent of choice to prevent infections due to *P. malariae*, *P. ovale* and *P. vivax* (Scheibel, 1997). For the prophylaxis in areas where malaria is endemic, mefloquine is the drug of choice, with doxycycline**,** the usual alternative for those who cannot take mefloquine (Grimberg and Mehlotra, 2011). Quinine is the drug of choice for the treatment of chloroquine-resistant *P. falciparum* infections. To improve compliance and maintain its efficacy, quinine is usually combined with tetracycline or doxycycline. For multidrug-resistant falciparum malaria, quinine is also given together with other blood schizonticides, such as antifolates or tetracyclines. For severe malaria, quinine or quinidine is given by intravenous injection (Khanna, 2007).

Artemisinin analogues, in particular artesunate and artemether, have recently shown great promise as rapidly acting and potent antimalarials (Katzung, 2006). Artemisinin-based combination therapies (ACTs) are recommended for treating falciparum malaria in all countries where resistance to monotherapies or non-artemisinin combination therapies (e.g. Artemether-lumefantrine (AL)) is prevalent (WHO, 2011). The rationale for the use of ACTs is based on the facts that artemisinin derivatives are highly potent and fast acting, and the partner drug has a long half-life, which allows killing the parasites that may have escaped the artemisinin inhibition (Grimberg and Mehlotra, 2011). In Ethiopia artemether-lumefantrine (Coartem) is used nationwide as the first line treatment for *P. falciparum* malaria (HSDP, 2010). However, the ever-increasing resistance of malarial parasites to the commonly available drugs necessitated the search for new drugs (Mesfin *et al*., 2012) which are inexpensive and routinely available (Abdulelah *et al.*, 2007). One approach to this is investigation of drugs from medicinal plants.

1.4. Medicinal Plants for Malaria Treatment

Plants have been used as a source of medicine throughout history and continue to serve as the basis for many pharmaceuticals used today (Ginsburg and Deharo, 2011). Traditional medicines are often more available and affordable, and sometimes are perceived as more effective than conventional antimalarial drugs (Alshawsh *et al.*, 2009). As shown in Appendix 1, there are numerous reports in the literature concerning the folklore use of medicinal plants as antimalarial agents; examples include *Cardiospermum halicacabum* L. (Sapindaceae), *Momordica foetida* Schumch. Et Thonn (Cucurbitaceae) (Waako *et al.*, 2005), *Acalypha fruticosa L.* (Euphorbiaceae), *Azadirachta indica* A. Juss (Meliaceae), *Boswellia elongata Balf* (Burseraceae), *Cissus rotundifolia Vahl* (Vitaceae), *Dendrosicyos socotrana Balf.* (Cucurbitaceae), *Echium vulgare* L (Boraginaceae) (Alshawsh *et al*., 2009), *Acanthospermum hispidum* (Asteraceae), and *Acokanthra schimperi* (Apocynaceae) (Sanon *et al*., 2003).

1.5. Ethiopian Medicinal Plants Used for Malaria Treatment

Ethiopia is a home of many languages, cultures and beliefs which in turn have contributed to the high diversity of traditional knowledge and practices of the people which, among others, include the use of medicinal plants (Giday *et al*., 2003). 80% of the Ethiopian population uses traditional medicine due to the cultural acceptably of healers and local pharmacopeias and the relatively lower cost of traditional medicine than modern drugs (Kassaye *et al*., 2006).

A number of studies have been conducted on the *in vitro* and *in vivo* evaluation of the anti-malarial activity of Ethiopian traditional medicinal plants (Dikasso *et al*., 2006) for instance, *Gnidia stenophylla*, *Vernonia bipontini*, *Euclea scimperi*, *Cissampelos*

mucronata, *Clerodendrum myricoides* (Assefa *et al*., 2007) *Dodonea angustifolia* and *Aloe debrana* (Deressa *et al*., 2010), *Combretum molle* (Aseres and Balcha, 1998), *Asparagus africanus* (Dikasso *et al*., 2006), *Aloe species*, *Azadirachta indica*, *Tamarindus indica* (Mesfin *et al*., 2011), *Gardenia ternifolia* Schumach & Thonn. (Rubiaceae), *Vernonia amygdalina* Del (Asteraceae) (Giday *et al*., 2009) have been shown to possess antimalarial activity.

Previous ethnobotanical studies revealed that several plant species contain antimalarial compounds (Mesfin *et al*., 2011).These include *Kniphofia foliosa* (Asphodelaceae) (Wube *et al*., 2005), *Warburgia ugandensis* (Canellaceae) (Wube *et al*., 2010) and *Combretum molle* (Combretaceae) (Asres *et al*., 2001).

1.6. The Genus *Aloe*

The name *Aloe* is derived from the Arabic word *alloeh,* which means a shining bitter substance in reference to the exudates (Dagne *et al.,* 2000). The genus *Aloe* comprises about 600 species, most of which are native to South Africa, the Saudi Arabian Peninsula, and to many islands of the western Indian Ocean, including Madagascar (UCDAVIS, 2009). Ethiopia has 40 species of *Aloe*, of these 20 species including *A. sinana, A. ankoberenesis,* and *A. harlana* are endemic (Bekele, 2007).

Aloe plants are easily recognized by their rosettes of large, thick, D-shaped/V-shaped succulent leaves, which are sometimes spotted. The leaf margin is almost always armed with sharp teeth. The inflorescence is usually branched, tubular shaped and their coloration is most often red, orange or yellow; rarely white (Demissew and Nordal, 2003). The flowers are held on single or branched stalks, and the resulting seeds are held in dry capsules (UCDAVIS, 2009).

Figure 2: The distribution of the genus *Aloe* (shown in orange) (UCDAVIS, 2009).

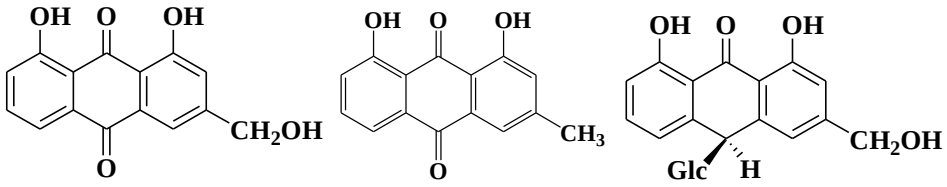
1.6.1. Ethnobotany of *Aloe*

A number of ethnobotanical uses of *Aloe* species have been recorded. A large number of these species is used throughout the world in traditional medicine. For example, leaves of *Aloe* species are widely used to treat cutaneous bacterial infections, as an antispasmodic and to relieve discomforts associated with menstruation in the Mascarene Islands (Sanmukhiya *et al*., 2010). *A. littoralis* and *A. vera* which are commonly known in Iran as "Sabre Zard", "Sebr" or "Segel", have been used in Iranian traditional and folk medicine to treat several disorders including dermatological, gastrointestinal and inflammatory diseases (Hajhashemi *et al*., 2012). *A. microdonta*, a species found in Somali is used as traditional medicine to treat jaundice and skin diseases (Farah *et al*., 1992, Viljoen *et al*., 2001). *A. excelsa* is used extensively as a traditional remedy in South Africa (Coopoosamy and Magwa, 2007). *A. macrocarpa* is used to treat eye disease and hemorrhoids in Bale Mountains National Park, Southeastern Ethiopia (Yineger *et al*., 2008).

1.6.2. Pharmacological Activity

1.6.2.1. Antimicrobial Activity

The methanol extract of *Aloe ferox* has been reported to show activity against six strains of *Neisseria gonorrhoea* (Kambizi and Afolayan, 2008). Aloe emodin (1), chrysophanol (2) and aloin (3) which were isolated from this plant exhibited promising antibacterial activity against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Shigella sonnei* (Kambizi *et al*., 2004). According to Mehrotra *et al*. (2010), *A. barbadensis* was found to inhibit the growth of methicillin resistant *Staphylococcus aureus*, *Vibrio chloerae* and *P. aeruginosa*. This plant showed the strongest action against *S. epidermidis* and *S. pyogenes* (Mehrotra *et al*., 2010; Bashir *et al.*, 2011).

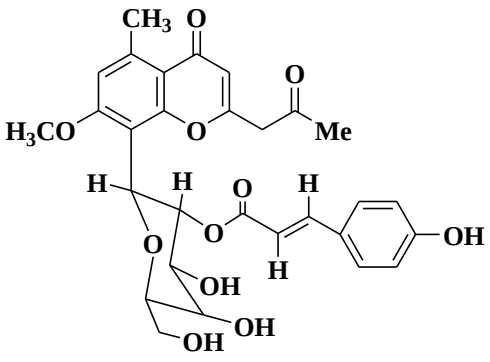


1 **2** **3**

1.6.2.2. Antioxidant Activity

An antioxidant is a substance that significantly delays or inhibits oxidation of the oxidizable substrate at low concentrations (Wamer *et al*., 2003). The chloroform-ethanol fraction of *A. vera* has been reported to possess strong radical scavenging activity in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sonia and Mohamed, 2008). Grace *et al.* (2010), studied free radical scavenging effects of the leaves of *A. arborescens* Miller (Kidachi aloe in Japanese) generated by streptozotocin (Sz). The results showed that boiled leaf skin of Kidachi aloe showed higher radical-scavenging activity than the non-boiled leaf skin powder. Aloin **(3)** isolated from this plant exhibited potent antioxidant activity (Arun *et al*., 2012). 7-O-methylaloeresin **(4)**,

which was isolated from *A. harlana* showed strong antioxidant activity (IC_{50} = 0.026 mM) in DPPH assay (Asamenew *et al*., 2011).



4

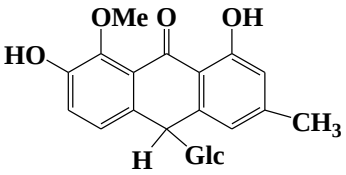
1.6.2.3. Antiinflammatory Activity

Mwale and Masika (2010) studied the antiinflammatory activity of aqueous leaf extract of *A. ferox*. The result showed that the plant significantly inhibit carrageen induced rat paw oedema at a dose of 400 mg/kg. Similarly, *A. littoralis* gel exhibited promising antiinflammatory activity in carrageenan test (Hajhashemi *et al*., 2012). Hydro-alcohol extracts of *A. buettneri* were tested on formaldehyde induced rat paw oedema and the extracts showed inhibition of oedema in a dose-dependent manner. This effect was proposed to be that the extract inhibits the production of bradykinin and substance P (Metowogo *et al*., 2008). Substance P, bradykinin, histamine, serotonin and prostaglandins are mediators of oedema and pain induced by formaldehyde (Amerash *et al*., 2007).

1.6.2.4. Antimalarial Activity

The methanol extract of the leaves of *A. debrana* induced 73.95% parasitaemia suppression against *P. berghei* in mice (Deressa *et al*., 2010). The dichloromethane/methanol extract of *A. ferox* showed anti-plasmodial activity with an

IC_{50} = 8 mg/mL (Clarkson *et al*., 2004). Pillay (2008), reported that homonataloin **(5)** possess higher activity than aloin against *Plasmodium falciparum* in *in vitro* assay.



5

1.6 3. Phytochemistry

The leaves and roots of *Aloe* species are store houses of many interesting secondary metabolites belonging to different classes of compounds including alkaloids, anthraquinones, pre-anthraquinones, anthrones, bianthraquinoids, chromones, flavonoids, and coumarins (Dagne, 1996). In Appendix 2, major classes of compounds which have been isolated from *Aloe* species are listed. In addition, the gel of *A. vera* contains inorganic minerals (ions of calcium, magnesium, zinc, iron, copper etc.), saccharides (arabinose, galactose, glucose, mannose and xylose), some amino acids, vitamins (B_1, B_2, B_6, B_{12}, C, etc.) and enzymes such as amylase, lipase, folic acid etc. (Dagne *et al*., 2000).

1.6.4. *Aloe sinana* Reynolds

Aloe sinana is one of the 20 endemic *Aloe* species of Ethiopia. It is locally known as "Rate", and grows on basaltic slope in Wello and Debre Sina, northeastern of Shewa floristic regions (Demissew and Nordal, 2003). Local People use leaves of this plant as antimalarial, wound healing, insecticides and for snake bite (Personal communication).

To the best of our knowledge, there is no report in the literature concerning the phytochemical and biological activities of *A. sinana*. In the present study the leaf latex

of the plant and the compounds isolated thereof have been investigated for *in vitro* antimicrobial and *in vivo* antimalarial activities based on the ethinomedicinal application of the plant.

Figure 3: Morphological view of *Aloe sinana*.

1.7. Statement of the Problem

Despite a control program lasting over 40 years 68% of the total population, is still at risk of infection with malaria. Besides individuals suffering caused by malaria, the agriculture dependent economy of Ethiopia is being weakened by the burden of this devastating disease. It is the leading cause of outpatient visits and it affects all age groups (DFID, 2011). Moreover, malaria is one of the diseases that accounts for 90% of child death (HSDP, 2010).

Limited number of antimalarial drugs and rapid emergence of drug resistance strains of the parasite worsen the disease in different regions of Ethiopia. These problems outpace the development of new antimalarials with novel modes of action. One approach to this is searching antimalarials from traditionally claimed plants from the Ethiopian flora.

The biodiversity of Ethiopian flora offers great possibilities in the search for novel compounds with antimalarial activity (Asres *et al.*, 2001). However, there is a need for scientific validation, standardization and safety evaluation of these plants before they are recommended for treatment of malaria.

In context with the above assessment, the present study has been conducted as a part of the ongoing research in the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University in the search for antimicrobial and antimalarial compounds from Ethiopian traditional medicinal plants.

2. OBJECTIVE OF THE STUDY

2.1. General Objective

- To evaluate the antimalarial and the antimicrobial activities of the latex of *Aloe sinana* Reynolds and the compounds isolated thereof.

2.2. Specific Objectives

- To investigate the latex of *Aloe sinana* for its antimalarial activity;
- To test the latex of *Aloe sinana* for its antimicrobial activity;
- To isolate and characterize compounds from the latex, and
- To determine the antimalarial and antimicrobial activities of the isolated compounds.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant Material

The leaf exudates of *Aloe sinana* was collected from Debre Sina in November, 2011. The authenticity of the plant material was confirmed by Professor Sebsebe Demissew, National Herbarium, Department of Biology, Addis Ababa University, where voucher specimen GM 02 was deposited.

3.1.2. Chemical, Reagent and Drugs

The following chemicals, reagents and drugs were used to perform the experiments: ethanol, chloroform, methanol, silica gel for thin layer chromatography $F_{254,}$ Giemsa, tween 80, hydrochloric acid, chloroquine phosphate (Ethiopian Pharmaceutical Manufacturing, Addis Ababa, Ethiopia), and ethyl acetate. All the chemicals were analytical grade and most of them were purchased from Pharmaceutical Fund and Supply Agency, Addis Ababa, Ethiopia, while the rest were obtained from the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University (AAU).

3.1.3. Instruments

Bruker Avance DMX400 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard at the Department of Chemistry, Collage of Natural Sciences, in Addis Ababa University, Ethiopia. Infra red (IR) spectra were determined on a Perkin-Elmer BX spectrometer (400-4000 cm^{-1}). Ultraviolet-Visible (UV-Vis) spectra were obtained using Shimadzu UV 1800 spectrometer. Electron spray ionization-mass spectrometry (ESI- MS) was performed by liquid chromatography (LC) coupled with mass spectrometry (MS).

3.1.4. Experimental Animals

Swiss albino mice of either sex, weighing 23-32 g and age of 6-8 weeks, were used in the study. The mice were obtained from the animal house of Biology Department at AAU. They were fed with standard commercial pellet food and tap water.

3.1.5. *Plasmodium berghei*

Chloroquine sensitive strains of *Plasmodium berghei* ANKA strain maintained at the animal house of Biology Department, AAU were used. The parasites were maintained by serial passage of blood from infected mice to the non-infected ones on weekly basis. Blood samples taken from donor mouse with the growing parasitaemia of 27-37% was diluted with normal saline, so that each 0.2 ml of blood contained 10^6-10^7 infected erythrocytes, the standard inoculums. These parasites were used to infect the experimental animals intraperitoneally (ip).

3.1.6. Microorganisms

The antibacterial was carried out against the following Gram-positive bacterial strains: *Bacillus subtilis* ATCC 6633, *B. pumillus* 82 and *Staphylococcus aureus* ML 267, and the Gram-negative bacterial strains used were: *Escherichia coli* K99, *E. coli* K88, *E. coli* CD/99/1, *E. coli* LT37, *E. coli* 306, *E. coli* 872, *E. coli* ROW 7/12, *E. coli* 3:37C, *Salmonella typhi* Ty2, *Shigella boydii* D13629, *S. flexneri* Type 6, *S. soneii* 1, *S. dysentery* 8, *Vibrio cholerae* 85, *V. cholerae* 293, *V. cholerae* 1313 and *V. cholerae* 1315. All the bacterial strains were procured from the Department of Technology, Jadavpur University; Central Drugs Laboratory, Kolkata and the Institute of Microbial Technology, Chandigarh, India. The stains were first checked for purity on the basis of standard microbiological, cultural and biochemical tests and then used for their sensitivity towards the test samples.

Antifungal activity testing was carried out on the following fungal pathogens: *Aspargillus niger* ATCC 6275, *Candida albicans* ATCC 10231, *Penicillum funiclosum* NCTC 287 and *P. notatum* ATCC 11625. All the fungal strains were procured from Central Drugs Laboratory, Kolkata, India.

3.2. Methods

3.2.1. Preparation of Plant Material

The latex was collected by cutting the leaves transversally near the base and inclining on stainless tray. The water was allowed to evaporate upon leaving the latex in open air for two days, which yielded a reddish dark substance.

3.2.2. Chromatographic Techniques

3.2.2.1. Preparative Thin Layer Chromatography (PTLC)

Isolation of compounds were performed by dissolving the latex in methanol and applied directly to preparative thin layer chromatographic plates (20 cm×20 cm) over silica gel of 0.5 mm thickness. The isolated compounds were purified by repeated preparative PTLC of 0.25mm thickness.

3.2.2.2. Solvent System

The solvent system used for both analytical and PTLC was a mixture of chloroform and methanol in the ratio of 4:1.

3.2.2.3. Visualization

The chromatographic zones were visualized first in daylight and then by using ultraviolet light of wave length 254 and 366 nm. After visualization the chromatographic zones were coded as AS_1, AS_2 and AS_3 based on descending order

of R_f values. Then, each band was carefully scrapped off separately from the plate and dissolved in methanol and chloroform (1:1), filtered and concentrated.

3.2.3. Spectroscopic Techniques

3.2.3.1. NMR, IR, UV and MS

NMR spectra were recorded on Bruker Avance DMX 400 FT-NMR spectrometer instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature using deutrated methanol. A region from 0 to 12 ppm for ^{1}H and 0 to 205 ppm for ^{13}C was employed for scanning. Signals were referred to an internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units and coupling constants (*J*) in Hz. Multiplicities of ^{1}H NMR signals are indicated as *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet), *m* (multiplate) and *nr* (not resolved). IR spectra were recorded with in the region between 400-4000 cm^{-1} in KBr pellets, since all samples are solids. UV spectra were recorded between 200-400 nm at room temperature. ESI-MS were recorded on Ultimate 3000 LC-MS. The measurement was carried out by an electrospray ionization method with negative mode. The source voltage and temperature were fixed at 3kV and 250 ºC.

3.2.4. Acid Hydrolysis

Hydrolysis experiments were carried out by dissolving each of the isolated compounds in 2% methanolic HCl and stirred for 12 h. The reaction progress was monitored by TLC and the reaction was stopped when the initial compound was completely converted to yellow coloured hydrolytic product. The reaction product was finally purified by PTLC.

3.2.5. Acute Oral Toxicity Test

Female Swiss albino mice were used for acute oral toxicity study. Oral toxicity study

was conducted as per the internationally accepted protocol drawn under OECD guidelines 425 (OECD, 2008). For each test samples ten mice were used and randomly divided in to 2 groups of 5 mice per cage. Before oral administration of a single dose of the test samples, the mice were deprived from food for 3 h. Then the mice in the first group were given latex of *A. sinana*, AS_1, AS_2 or AS_3 orally at a dose of 2000 mg/kg by using gavage. The mice in the second group were given the test samples orally at a dose of 5000 mg/kg. Each test sample was dissolved in distilled water except AS_1 which was dissolved by vehicle (distilled water: ethanol: tween-80 in the ratio of 90:3:7, respectively).

The mice were observed continuously for the first 30 min. after administration of the test sample; intermittently for 4 h, over a period of 24 h and for 14 days. Gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality and other signs of toxicity manifestation were observed (OECD, 2008).

3.2.6. Determination of Antimicrobial Activities

3.2.5.1. Antibacterial Assay

The zone of inhibition produced by the test samples was determined and compared with that of ciprofloxacin by a disc diffusion method (Mitchell and Carter, 2000). Two sets of dilution of 200 μg/mL, each of the test samples dissolved in dimethyl sulphoxide (DMSO) and ciprofloxacin (dissolved in sterile distilled water) were prepared in sterile McCartney bottles. Serial nutrient agar plates were prepared and incubated at 37 ºC for 24 h to check for any sort of contamination. Sterile filter paper discs (Whatman no. 1) of 6 mm diameter were soaked in stock solution (200 μg/mL) of test samples and placed in appropriate position on the surface of the flooded plate

seeded with 24 h old culture grown on nutrient broth, marked as quadrant at the back of the Petri dishes. The Petri dishes were then incubated at 37 ºC for 24 h and the diameter of zone of inhibition were measured in mm. Similar procedure was adopted for the pure ciprofloxacin and zone of inhibition was compared accordingly. DMSO was used as a negative control.

3.2.5.2. Antifungal Assay

The antifungal potential of the test samples (2000 µg/mL) was evaluated by disc diffusion method (as described for the determination of antibacterial activity) against the fungal pathogens on Saborauds dextrose media. The Petri dishes were incubated at room temperature for 3 days and the diameter of zone of inhibition was measured in mm. The antifungal agent, griseofulvin was used as a reference standard.

3.2.5.3. Minimum Inhibitory Concentrations (MIC)

Minimum inhibitory concentrations (MIC) of the latex and the isolated compounds were determined by the method described by Hecht *et al*. (2006). Nutrient agar and Saborauds dextrose agar were used for bacterial and fungal growth, respectively. Concentrations of 5, 10, 25, 50, 100, 200, 400 and 800 µg/mL for antibacterial activity testing and 50, 100, 200, 400, 800, 1000, 1500 and 2000 µg/mL for antifungal activity testing, of the latex and the isolated compounds dissolved in DMSO were used. A sterility control was also carried out (growth control contained nutrient broth plus DMSO, without antimicrobial substances). Each test and growth control well was incubated at 37 ºC for bacteria and 25 ºC for fungi.

3.2.7. Antimalarial Activity Test

3.2.7.1. Antimalarial Activity Test of the Latex and Isolated Compounds

Plasmodium berghei 4 day suppression test was used to evaluate the *in vivo* antimalarial activity of the latex and isolated compounds using Swiss albino mice (Kalra *et al*., 2006). The mice were housed in standard cages and maintained on standard pellated diet and water for 5 days, to the laboratory conditions. Blood was taken from a donor mouse with approximately 27-37% parasitemia and diluted in physiological saline to $2x10^7$ parasitized erythrocytes per mL. Male Swiss albino mice weighing 23-32g were infected with 0.2 mL (about $2x10^7$ parasites) *P. berghei* i.p. and randomly divided into five groups of five mice per cage with three test groups and two control groups (one for chloroquine as a positive control and the other distilled water or vehicle as a negative control) for each test sample.

The latex and isolated compounds (AS_1 and AS_2) were prepared at different doses of 100, 200 and 400 mg/kg of body weight and chloroquine at 25 mg/kg in a volume of 0.5 mL. AS_3 was prepared at lower doses of 25, 50 and 100 mg/kg of body weight. The test samples or the standard were administered as a single dose per day and given through oral route by using standard oral gavage. Treatment was started 3 h post infection on day 0 and was then continued daily for four days (i.e. from day 0 to day 3). Mice that were administered with latex, AS_2 or AS_3 received distilled water as a negative control, whilst vehicle containing a solution of 7% Tween 80 and 3% ethanol was given to the mice that took AS_1.

On the fifth day (D4), each mouse was weighed and thin smears of blood films were obtained from the tail. The smears were placed on microscopic slides (Westtmed Praxis, Germany), fixed with methanol and stained with 10% Giemsa for 20 min.

Parasitaemia level was determined by counting the number of parasitized erythrocytes out of six random fields of the microscope (Olympus 6V20WHA2, Japan). Average percent parasitaemia and suppression were calculated using the following formula (Kalra *et al*., 2006).

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC count}} \times 100$$

$$\% \text{ suppression} = \frac{\text{Mean parasitemia of negative control} - \text{Mean parasitemia of treated}}{\text{Mean parasitemia of negative control}} \times 100$$

3.2.8. Data Analysis

Results were presented as a mean plus or minus standard error of the mean (M ± SEM) by using computer software SPSS version 20. The one-way analysis of variance (ANOVA) followed by Tukey's HSD *post-hoc* test, were used to compare results among and within groups for difference between initial and final results. Paired *t-test* was also used to compare some parameters between initial and final results. The results were considered significant when $P<0.05$.

4. RESULTS AND DISCUSSION

4.1 Leaf Latex of *A. sinana*

4.1.1 Acute Toxicity

No sign of toxicity or mortality was observed in mice up on oral administration of the latex of *A. sinana*, up to the highest dose of 5000 mg/kg, signifying that the oral LD_{50} is greater than 5000 mg/kg. Toxicity is the main concern of indigenous therapeutic preparations (Jayasinghe *et al*., 2008). The fact that changes in general behaviour, effect on body weight and mortality, which are critical for the evaluation of adverse effects, were not evident on the test animals which is good evidence for the absence of toxicity. This fulfils the criteria set by OECD (2008) guideline for lack of acute toxicity. Therefore, the latex may be considered to be relatively safe in mice when given orally suggesting the safety of the plant when used by the local people.

4.1.2. Antimicrobial Activity

4.1.2.1. Antibacterial Assay

The *in vitro* antibacterial activity of the latex of *A. sinana* against the employed bacteria was assessed by using the disk diffusion method. As shown in Table 1, at a concentration of 200 µg/mL, the latex exhibited potent inhibitory effect against the tested bacterial pathogens. The Gram-negative bacteria including all strains of *E. coli*, *S. typhi* (Ty2), *Shigella* spp. and *V. cholerae* strains were found to be the most inhibited bacterial pathogens by the latex. The latex also showed strong antibacterial activity against the Gram-positive strain *S. aureus* ML 267. Gram-positive bacteria: *Bacillus pumilus* 82 and *B. subtilis* ATCC 6633 were inhibited moderately compared with the reference drug ciprofloxacin. In general, the activity of the latex was comparable to that of the standard ciprofloxacin.

Table 1: Diameter of zone of inhibition and minimum inhibitory concentration (MIC) of the latex of *Aloe sinana* in comparison with that of ciprofloxacin.

Microorganism	Diameter of zone of inhibition in mm. (µg/mL)		MIC (µg/mL)
	Latex	Ciprofloxacin	Latex
Bacillus pumilus 82	12.0 (63.2)	19.0	100
B. subtilis ATCC 6633	11.0 (61.1)	18.0	100
Escherichia coli CD/99/1	15.5 (91.2)	17.0	25
E. coli K88	15.0 (88.2)	17.0	25
E. coli K99	15.5 (96.9)	16.0	25
E. coli LT37	14.5 (90.6)	16.0	25
E. coli ROW 7/12	14.5 (87.9)	16.5	25
E. coli 3:37C	14.5 (93.5)	15.5	25
E. coli 306	15.0 (90.9)	16.5	25
E. coli 872	14.5 (90.6)	16.0	25
Salmonella typhi Ty2	14.0 (87.5)	16.0	25
Shigella boydii D13629	17.5 (87.5)	20.0	10
S. dysentery 8	15.5 (73.8)	21.0	10
S. flexneri Type 6	17.5 (85.4)	20.5	10
S. soneii 1	17.0 (87.2)	19.5	10
Staphylococcus aureus ML267	14.5 (80.6)	18.0	50
Vibrio cholerae 85	14.5 (80.6)	18.0	25
V. cholerae 293	15.0 (85.7)	17.5	25
V. cholerae 1313	15.5 (91.2)	17.0	25
V. cholerae 1315	15.0 (83.3)	18.0	25

Figures in parenthesis indicate % activity of the test samples compared with that of ciprofloxacin.

4.1.2.2. Minimum Inhibitory Concentration (MIC)

The present results revealed that the leaf latex of *A. sinana* was much more active. As shown in Table 1, the latex of *A. sinana* exhibited broad spectrum antibacterial activity against the bacterial strains tested. It displayed strong effect against all the tested Gram-negative bacterial strains of *Shigella* and *E. coli* with MIC values of 10 and 25 µg/mL, respectively. Gram-positive bacterial strains *B. pumilus* 82 and *B. subtilis* ATCC 6633 were moderately inhibited by the latex with MIC value of 100 µg/mL.

The present results revealed that the leaf latex of *A. sinana* was much more active against the Gram-negative bacteria than the Gram-positive ones. These results are similar with those obtained for the latex of *A. harlana* (Asamnew *et al*., 2011).

Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to resistant strains (Grover *et al*., 2011). This has led to an urgent global call for new antimicrobial drugs, particularly from natural resources. These results indicated that the potential antibacterial activity of the leaf latex of *A. sinana* against the tested bacterial pathogens and could be promising for further antibacterial study.

Previous studies concerning the *in vitro* antimicrobial activity of *Aloe* spp. indicated that *Aloe* extracts possess antimicrobial activities against both Gram-positive and Gram-negative bacteria including *S. aureus*, *E. coli*, and *Klebsiella pneumonia* (Ndhala *et al*., 2009). This is majorly due to the presence of pharmacologically active compounds including anthraquinones such as homonataloin, aloe emodin, aloin (the C-glucoside of aloe emodin), chrysophanol etc, which have been reported to exhibit

antimicrobial activity (Hamman, 2008; Kambizi and Afolayan, 2008). Therefore, the antibacterial activities of leaf latex of *A. sinana* might be due to the presence of anthraquinones.

4.1.2.3. Antifungal Activity

The latex of *A. sinana* was also assessed by disk diffusion method against four fungal strains, namely *A. niger* ATCC 6275, *C. albicans* ATCC 10231, *P. funiculosum* NCTC 287 and *P. notatum* ATCC 11625. As shown in Table 2, the activity of the latex was compared with that of the standard reference drug griseofulvin against the fungal strains tested. The latex exhibited moderate activity against *A. niger* and *C. albicans* with MIC value of 1000 µg/mL, while less activity was observed against the two *Penicillum* species namely, *P. funiculosum* and *P. notatum* with (MIC= 2000 µg/mL).

Table 2: Antifungal activity of the leaf latex of *Aloe sinana.*

Microorganism	Diameter of zone of inhibition in mm (2000 µg/mL)		MIC (µg/mL)
	Latex	Griseofulvin	Latex
Aspaergillus niger ATCC 6275	11.0(73.3)	15.0	1000
Candida albicans ATCC 10231	11.5(71.9)	16.0	1000
Penicillium funiculosum NCTC 287	9.5(67.9)	14.0	2000
P. notatum (ATCC 11625)	9.5(70.4)	13.5	2000

Figures in parenthesis indicate % activity of the test samples compared with that of griseofulvin.
MIC: Minimum inhibitory concentration.

Fungal infections are the primary cause of mortality in patients with severely impaired host defense mechanisms, such as neutropenic patients with acute leukemia or those who have undergone bone marrow transplantation (Kullberg, 1997). Furthermore, the increase of AIDS related fungal infection and the emergence of resistance strains to

the current antifungal have lent additional urgency to studies on potential antifungal agent (James and Harrison, 2012). From the results of the present study it can be said that the leaf latex of *A. sinana* could be a potential antifungal agent.

4.1.3. Antimalarial Activity of Leaf Latex of *A. sinana*

As shown in Table 3, the latex of *A. sinana* possesses activity against *P. berghei* malaria parasite *in vivo*. The results also revealed that the latex has dose dependent activity. Thus, at doses of 100, 200 and 400 mg/kg/day, it caused 53.4, 62.3 and 68.2% suppression, respectively. The chemosuppression was statistically significant (P<0.001) when compared with the negative control. In the same assay, mice treated with chloroquine were completely free from the parasites on day four at the dose level of 25 mg/kg/day.

Table 3: Antimalarial activity of the latex of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei*.

Test substance	Dose mg/kg/day	% Parasitaemia ± SEM	% Suppression	Survival time ± SEM
Vehicle	NC	23.6 ± 0.51	-	7 ± 0.32
Latex	100	11.0 ± 0.16*	53.4	8 ± 0.37
Latex	200	8.9 ± 0.19*	62.3	10 ± 0.24*
Latex	400	7.5 ± 0.16*	68.2	10 ± 0.20*
Chloroquine	25	00.00*	100	12 ± 0.20*

*Mean value is significant (P<0.001) when compared with vehicle treated group; Values are presented as mean ± SEM; n=5; NC=negative control.

From the results shown in Table 3, it is evident that the latex of *A. sinana* possesses blood schizontocidal activity in the early infection of mice with *P. berghei* parasite. The average percent parasitemia observed at a higher dose (400 mg/kg/day) was

found to be 7.5 ± 0.16% with 68.2% of chemosuppression. A dose of 200 mg/kg/day of the latex showed 8.9 ± 0.19% parasitemia with 62.3% chemosuppression, while the lowest dose (100 mg/kg/day) exhibited a higher percent parasitemia 11.0 ± 0.16 with lowered chemosuppression of 53.4%.

The mean survival time of mice that were fed with the latex was 8 ± 0.37, 10 ± 0.24 and 10 ± 0.20 days, for doses of 100, 200 and 400 mg/kg/day, respectively. The mean survival time were statistically significant (P<0.001) except for the lower dose (100 mg/kg/day) when compared to vehicle treated mice. Furthermore, although statistically not significant, the mean survival time of mice treated with 100 mg/kg/day of the latex was relatively longer than the negative control. The latex showed significant effect on the mean survival time of the treatment groups compared to the untreated control. This effect has value in patient care whereby the latex could be used until such time that curative medication can be found for proper treatment of malaria patients.

Treatment of *P. berghei* infected mice with the latex of *A. sinana* also prevented them from weight loss. The mean weight of each group improved on the fifth day of infection. The improvement was not statically significant (P>0.05). However, all treated group showed weight increment when compared to the negative control (Table 4). The much higher increment of body weight by the latex compared even to that of chloroquine might be due to an appetite inducing effect of the latex in addition to antimalarial active compounds. This effect was also seen in acute toxicity tests which resulted in slight increment of body weight in latex treated groups. These result clearly indicated that leaf latex of *A. sinana* prevented weight loss in a dose dependent manner compared to the controls.

Table 4: Body weight of *Plasmodium berghei* infected mice after administration of the latex of *Aloe sinana.*

Test substance	Dose mg/kg/day	Weight D0 ± SEM	Weight D4 ± SEM	% Change
Vehicle	0.5ml	25.28 ± 0.28	24.4 ± 0.3	-3.6
Latex	100	25.40 ± 0.65	25.7 ± 0.57	1.1
Latex	200	26.60 ± 0.55	27.6 ± 0.82	3.6
Latex	400	26.21 ± 0.63	27.6 ± 0.65	5.0
Chloroquine	25	30.29 ± 0.16	31.4 ± 0.96	3.5

Data are expressed as mean ± SEM for five mice per group; Weight D0: Weight pre-treatment on day zero; Weight D4: weight post-treatment on fifth day.

Literature survey reveals that several plants of the genus *Aloe* possess antimalarial activity. For instance, the methanol extract of *A. debrana* leaves induced 73.95% parasitaemia suppression at a dose of 600 mg/kg against *P. berghei* (Deressa *et al.*, 2010). In this study, the latex of *A. sinana* showed considerable antiplasmodial properties. Suppression increased with increase in the dose of the latex. The antiplasmodial activity of the latex could be due to the presence of anthraquinones or other quinoid compounds that are the characteristic constituents of the genus.

According to Deharo *et al.* (2001) an *in vivo* antiplasmodial activity can be classified as moderate, good, and very good if an extract displays percent parasite suppression equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg body weight per day, respectively. Based on this classification, the leaf latex of *A. sinana* exhibited moderate antiplasmodial activity.

4.2. Compounds Isolated from the Latex of *A. sinana*

4.2.1. Isolation and Structural Elucidation

Repeated preparative TLC of the leaf latex of *A. sinana* over silica gel using chloroform: methanol (4:1) as a solvent system, afforded three major compounds with R_f values of 0.57, 0.36 and 0.15. These compounds were designated as AS_1, AS_2 and AS_3, respectively, as shown in Figure 4. The compounds appeared bright yellow when viewed in daylight, dark under UV light of λ 254 nm and dark orange under UV 366 nm. Structural elucidation of the isolated compounds was based on their spectroscopic data and by comparison of their spectroscopic characteristics with those reported in the literature.

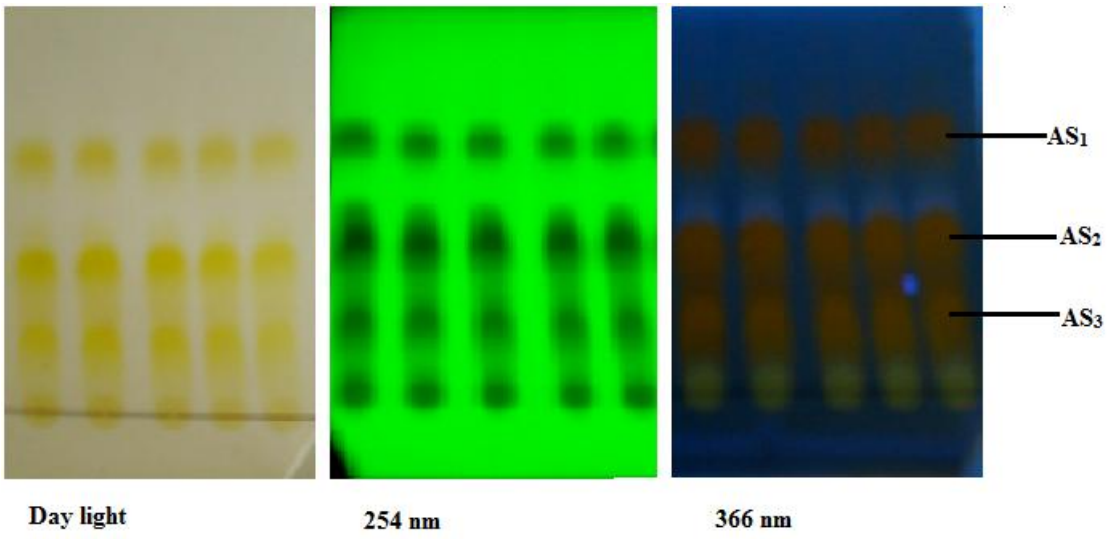


Figure 4: TLC of the compounds isolated from the leaf latex of *Aloe sinana* when viewed in day light and under UV light of λ 254 nm and 366 nm (solvent system chloroform: methanol 4:1, respectively).

4.2.1.1. AS_1

AS_1 was isolated as a yellow solid with R_f value of 0.57 in $CHCl_3$: MeOH (4:1). The negative-ion ESI – MS data of AS_1 gave a pseudomolecular ion at *m/z* 563 $[M - H]^-$, indicating a relative molecular weight (M_r) of 564, which was consistent with ^1H and ^{13}C-NMR data (Table 5). In the IR spectrum (Appendix 3), a broad absorption band at

3415 cm^{-1} and a strong absorption band at 1709 cm^{-1} indicated the presence of OH and carbonyl groups, respectively. Two medium peaks at 1297 cm^{-1} and 1168 cm^{-1} indicated the presence of C-O group. Peaks at 1603 cm^{-1} and 1453 cm^{-1} are indicative of C=C bond of aromatic ring (Appendix 3). The UV spectrum (Appendix 3), displayed an absorption band at λ_{max} (in MeOH), 302 and 311 nm indicating the presence of an anthrone moiety (Dagne *et al*., 2000).

The presence of two chelated hydroxyl groups was supported by the ^{1}H NMR spectrum (Table 5), which showed two singlets at δ 12.01 (1H, *s*, H-1) and δ 12.17 (1H, *s*, H-8), in addition to five aromatic protons assignable to H-2 (δ 6.91), H-4 (δ 7.28), H-5 (δ 7.09), H-6 (δ 7.5) and H-7 (δ 6.91). Furthermore two olefinic protons which were assignable to *trans*-vinyl H-8" (δ 5.92, *d*, *J* = 16 Hz) and H-7" (δ 7.24, *d*, *J* = 16 Hz) and four aromatic protons to H-2" & H-6" (δ 7.36, *d*, J = 8.2 Hz, 2H) and H-3" & H-5" (δ 6.82, *d*, J = 8.2 Hz, 2H) led to a *trans*-cumaroyl residue as part of AS_1.

The ^{13}C-NMR and DEPT-135 spectra (Appendix 3), showed signals for 30 different carbon atoms corresponding to two oxymethylenes, 16 methines and 12 quaternary carbon atoms including a chelated carbonyl (δ 194.80) and an ester carbonyl carbon (δ 167.73). In addition, the DEPT-135 spectrum displayed two downward peaks at δ 63.15 and δ 64.5, which showed the presence of two methylene groups attached to oxygen.

Acid hydrolysis of AS_1 gave a yellow product with R_f value 0.36 which was identified as aloin. This result indicated that there was a loss of coumaroyl moiety from the parent molecule. From the data presented above and by comparison of the ^{1}H and ^{13}C NMR data reported for a similar compound (Farah *et al*., 1992), AS_1 was identified as

microdontin.

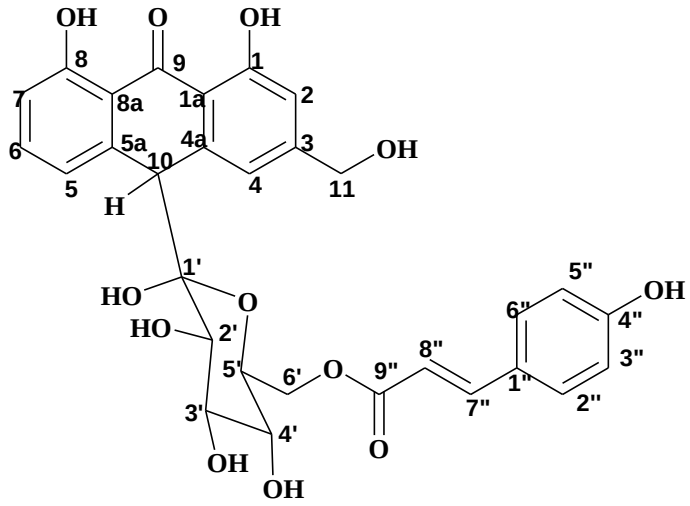


Figure 5: The structure of AS_1 (microdontin).

4.2.1.2. AS_2

AS_2, with R_f value of 0.36 in $CHCl_3$: MeOH (4:1), was obtained as a yellow amorphous solid. The negative-ion ESI-MS data of AS_2 showed a pseudomolecular ion at *m/z* 417, corresponding to M_r of 418. This together with ^1H and ^{13}C NMR data including DEPT was in agreement with the molecular formula $C_{21}H_{22}O_9$.

The IR spectrum of AS_2 exhibited absorptions consistent with a hydroxyl (3447 cm^{-1}), C=O stretching of unsaturated carbonyl group (1631 cm^{-1}) and C=C stretching of aromatic ring (1618 cm^{-1}). Compound AS_2 exhibited absorption maxima (208, 299 and 357) in its UV spectrum (Appendix 4) that are a typical of anthrone moiety (Dagne *et al*., 2000).

The ^1H NMR spectral data of AS_2 revealed the presence of two H-bonded phenolic OH singlets at 11.96 and 11.97 ppm. Moreover five aromatic signals were assigned to H-2 (δ 6.88, 1H, *s*), H-4 (δ 7.04, 1H, *nr*), H-5 (δ 7.06 1H, *d*), H-6 (δ 7.5 1H, *dd*) and H-7 (δ 6.87, 1H, *dd*). A singlet at δ 4.67 was also observed in the ^1H NMR due to the presence of a methylene group attached to oxygen (2H, *s*, H-11).

Table 5: ^{1}H and ^{13}C NMR spectral data of $AS_{1.}$

Assignments	H^{1} (δ, ppm)		^{13}C (δ, ppm)	
	AS_1	(Microdontin*)	AS_1	(Microdontin*)
1	12.01	-	163.78	163.7
2	6.91	6.88	114.21	114.5
3	-	-	152.43	152.3
4	7.28	7.25	118.8	118.8
5	7.09	7.07	121.1	121.0
6	7.5	7.49	137.1	137.0
7	6.91	6.87	117.3	117.3
8	12.17	-	163.67	163.6
9	-	-	194.8	194.72
10	4.55-4.59	4.5	46.5	46.5
1a	-	-	116.68	116.6
4a	-	-	143.81	143.8
5a	-	-	143.53	143.5
8a	-	-	117.4	117.4
11	4.69-4.74	4.63-4.72	64.5	64.5
1'	3.92	4.01	85.18	85.1
2'	4.36	4.38	72.9	72.9
3'	4.05	3.36	78.3	78.28
4'	3.11	3.08	71.5	71.5
5'	3.33	3.3	81.8	81.8
6'a	3.67	3.64	63.15	63.1
6'b	3.87	3.86		
1"	-	-	127.24	127.12
2"	7.36	7.35	131.31	131.2
3"	6.82	6.81	116.81	116.7
4"	-	-	161.25	161.2
5"	6.82	6.81	116.91	116.7
6"	7.36	7.35	131.31	131.2
7"	7.24	7.23	146.81	146.7
8"	5.92	5.89	114.67	114.7
9"	-	-	167.73	167.57

* Farah *et al*. (1992).

The ^{13}C-NMR and DEPT-135 spectra (Table 6 and Appendix 4) revealed 21 carbon atoms, which were identified as two oxymethylene (δ 63.12 and 61.82), five aromatic methine (δ 113.02, 115.42, 117.75, 118.89 and 135.66) and five oxymethine (δ 70.45, 70.56, 78.56, 85.21 and 78.56). Eight quaternary carbons were also identified in AS_2 from ^{13}C-NMR and DEPT-135 spectra, including signal due to a carbonyl ketone (δ 194.1). The structure of this compound was further confirmed as aloin (10-C-β-D-glucopyranosyl-1, 8-dihydroxymethyl-9-anthracenone) by comparing the NMR data with those reported in the literature for the same compound (Farah *et al*., 1992).

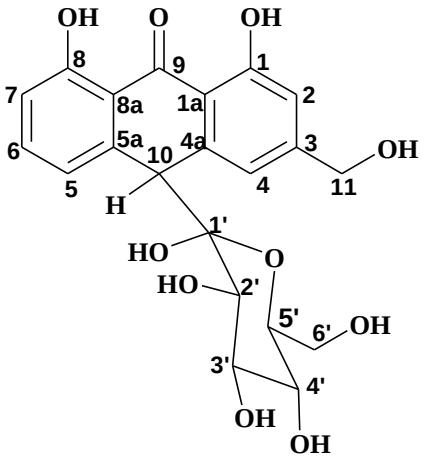


Figure 6: The structure of AS_2 (Aloin).

4.2.1.3. AS_3

AS_3 was also obtained as a yellow amorphous solid with R_f value of 0.15 in the solvent system $CHCl_3$: MeOH (4:1). The negative-mode of ESI-MS of AS_3 gave a pseudomolecular ion at *m/*z 563 $[M - H]^-$, indicating a relative molecular weight (M_r) of 564, which was in good agreement with ^{1}H and ^{13}C NMR data. As shown in Appendix 5, a strong broad IR absorption band at 3394 cm^{-1} and medium absorption band at 1634 cm^{-1} indicated the presence of an OH and a carbonyl group, respectively. Two sharp peaks at 1618 cm^{-1} and 1451 cm^{-1} indicated the presence of an aromatic group. An absorption band at 1288 cm^{-1} and 1076 cm^{-1} indicated that there is a C-O

Table 6: ^{1}H and ^{13}C NMR spectral data of AS_2.

Assignment	^{1}H (δ, ppm)		^{13}C (δ, ppm)	
	AS_2	(Aloin*)	AS_2	(Aloin*)
1	11.97	-	161.95	163.4
2	6.88	6.88	113.02	114.4
3	-	-	150.11	151.6
4	7.04	7.06	117.75	119.1
5	7.06	7.07	118.59	120.0
6	7.5	7.5	135.66	137.0
7	6.87	6.86	115.42	116.8
8	11.96	-	161.49	163.0
9	-	-	194.1	195.4
10	4.58	4.62	44.48	46.0
1a	-	-	117.75	117.7
4a	-	-	141.84	143.3
5a	-	-	145.15	146.6
8a	-	-	117.23	118.7
11	4.67	4.61-4.69	63.12	64.5
1'	3.41	3.42	85.21	86.7
2'	3.02	3.01	70.56	71.9
3'	3.28	3.25	78.56	81.7
4'	2.91	2.9	70.45	72.0
5'	2.93	2.92	80.26	80.0
6'a	3.36	3.38	61.82	63.30
6'b	3.56	3.57		

* Farah *et al*. (1992).

group in the compound. The UV spectrum (Appendix 5) revealed an absorption band at λ_{max} (in MeOH) 211, 222, 298 and 357 nm indicating the presence of an anthrone moiety (Dagne *et al*., 2000).

AS_3 exhibited a pattern of signals in the ^1H and ^{13}C NMR spectra (Tables 7) similar to that of AS_2, except for the presence of additional signals due to a rhamnose moiety (C-1" (δ 4.9, 100.7), C-2" (δ 3.9, 70.6), C-3"(3.9, 72.1), C-4" (δ 4.0, 68.8), C-5" (δ 4.5, 85.0) and C-6" (1.2, 17.8)). The pseudomolecular ions of AS_3 in the negative-mode ESI-MS appeared 146 amu higher than those observed in AS_2, suggesting the presence of an *O*-rhamnose unit in AS_3, an observation that was confirmed by conversion of AS_3 to AS_2 by acidic hydrolysis using methanolic HCl. The *O*-rhamnose unit in AS_3 was placed on C-11 oxygen based on the glycosilation shift of + 4.58 ppm relative to that for the same carbon in compound AS_2 (Table 6). Complete assignments of protons and carbons are listed in Table 7.

From the spectroscopic data obtained for AS_3 and comparison of the NMR chemical shifts those reported in the literature (Dagne and Alemu, 1991), the compound was identified as 10-glucopyranosyl-1, 8-dihydroxy-3-(rhamnopyranosyl-hydroxymethyl)-9 (10H) -anthracenone, commonly called aloinoside.

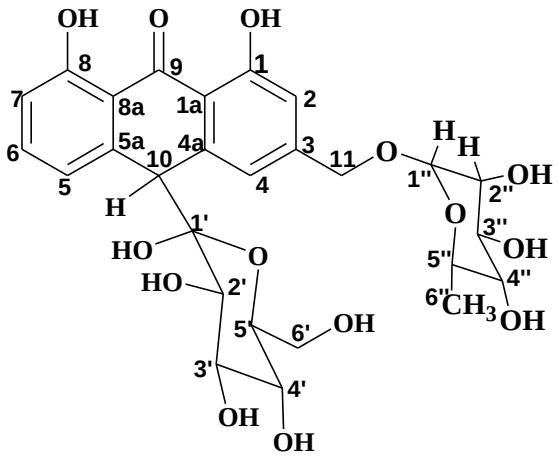


Figure 7: The structure of AS_3 (Aloinoside).

Table 7: ^{1}H and ^{13}C NMR spectral data of AS_3.

Assignment	H^1 (δ, ppm)		^{13}C(δ, ppm)	
	AS_3	(Aloinoside*)	AS_3	(Aloinoside*)
1	12.19	11.8	160.5	160.8
2	6.8	6.5	113.7	113.7
3	-	-	145.9	145.3
4	7.0	7.0	117.7	117.1
5	6.9	6.9	118.5	118.0
6	7.4	7.4	136.1	135.4
7	7.1	7.1	115.3	115.3
8	11.9	11.8	161.3	160.8
9	-	-	195.2	193.2
10	4.6	4.6	45.3	43.9
1a	-	-	114.6	114.0
4a	-	-	141.7	141.3
5a	-	-	146.4	145.2
8a	-	-	116.9	117.0
11	4.8	4.8	67.7	67.0
Glc 1'	3.3	3.3	77.9	78.0
2'	3.0		70.4	70.4
3'	3.0		79.6	79.5
4'	2.9	2.5 - 3.4	70.0	70.1
5'	2.7		85.1	84.7
6'a	3.4		61.85	61.7
6'b	-			
Rha 1"	4.9	5.1	100.7	99.4
2"	3.9		70.6	70.8
3"	3.9	3.5 - 4.9	72.1	72.4
4"	4.0		68.8	68.5
5"	4.5		85.0	84.7
6"	1.2	1.2	17.8	17.1

* Dagne and Alemu (1991).

4.2.2. Acute Toxicity of Isolated Compounds

No sign of toxicity or mortality was observed in mice after oral administration of the isolated compounds (microdontin, aloin and aloinoside) at a dose of 5000 mg/kg, signifying that their oral LD_{50} was greater than 5000 mg/kg. Lack of gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions,

salivation etc. on the test animals is good evidence for the absence of toxicity of the test compounds. This fulfills the criteria set by OECD (2008) for lack of acute toxicity. Therefore, these compounds were found to be non-toxic to mice, as they did not show signs of acute toxicity within 24 h at the dose levels up to 5000 mg/kg body weight.

4.2.3. Antimicrobial Activity

4.2.3.1. Antibacterial Assay

The results of the antibacterial activity study were compiled in terms of diameter of zone of inhibition in mm. All the tested compounds showed broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. As shown in Table 8, a strong activity was observed against all strains of *E. coli* and *V. cholerae,* which was comparable to ciprofloxacin.

Higher activities of the compounds were also observed against the *Shigella* strains tested and *S. typhi* Typ 2, a human pathogen which causes typhoid fever. The effect of all the isolated compounds was rather moderate against *S. enteric* TD 01 and weak against *Bacillus* species. The isolated compounds displayed potent action against *S. aureus*.

4.2.3.2. Minimum Inhibitory Concentration (MIC)

As shown in section 4.2.3.1, the isolated compounds exhibited broad spectrum antibacterial activity. The Gram-negative bacteria including all strains of *E. coli*, *S. typhi, Shigella* spp, and *V. cholera* were found to be the most inhibited bacterial pathogens by microdontin (Table 9). Aloin exhibited moderate activity against *S. aureus,* but a strong inhibitory effect against all strains of *E. coli*, *S. typhi*, *Shigella* spp, and *V. cholera* (MIC= 25 µg/mL). According to Asamenew *et al*. (2011), aloin

Table 8: Diameters of zone of inhibition of compounds isolated from leaf latex of *Aloe sinana* against some bacterial strains.

Microorganism	Diameter of zone of inhibition mm (200 µg/mL)			
	Microdontin	Aloin	Aloinoside	Ciprofloxacin
B. pumilus 82	9.5(50)	10.0(52.6)	6.0(31.58)	19.00
B. subtilis ATCC663	9.5(52.8)	10.0(55.6)	6.0(33.3)	18.00
Escherichia coli CD/99/1	17.0(100)	15.5(91.2)	15.5(91.2)	17.00
E. coli K88	15.5(91.2)	15.0(88.2)	15.0(88.2)	17.00
E. coli K99	16.0(100)	15.5(96.9)	15.5(96.9)	16.00
E. coli LT37	15.0(93.8)	14.5(90.6)	14.5(90.6)	16.00
E. coli ROW 7/12	14.5(87.9)	14.5(87.9)	14.5(87.9)	16.50
E. coli 3:37C	14.5(93.5)	14.5(93.5)	14.5(93.5)	15.50
E. coli 306	15.5(93.9)	15.0(90.9)	15.0(90.9)	16.50
E. coli 872	15.0(93.8)	14.5(90.6)	14.5(90.6)	16.00
Salmonella typhi Ty2	14.0(87.5)	14.0(87.5)	14.0(87.5)	16.00
S. enterica TD 01	12.5(65.8)	12.5(65.8)	12.5(65.8)	19.00
Shigella boydii D13629	16.5(82.5)	16.5(82.5)	17.5(87.5)	20.00
S. dysentery 8	15.5(73.8)	15.5(73.8)	15.5(73.8)	21.00
S. flexneri Type 6	16.5(80.5)	16.5(80.5)	17.5(85.4)	20.50
S. soneii 1	16.0(82.1)	16.0(82.1)	17.0(87.2)	19.50
Staphylococcus aureus ML267	17.0(94.4)	14.5(80.6)	16.5(91.7)	18.00
Vibrio cholerae 85	16.5(91.7)	14.5(80.6)	14.5(80.6)	18.00
V. cholerae 293	16.0(91.4)	15.0(85.7)	15.0(85.7)	17.50
V. cholerae 1313	16.5(97.1)	15.5(91.2)	15.5(91.2)	17.00
V. cholerae 1315	17.0(94.4)	15.0(83.3)	15.0(83.3)	18.00

Figures in parenthesis indicate % activity of the test sample compared with that of ciprofloxacin.

which was isolated from *A. harlana* also showed activity against *E. coli*, *S. typii* Ty2, *Shigella* and *V. cholera* bacterial strains. *B. pumilus* and *B. subtillis* were found to be the most resistant bacterial strains to aloinoside, whereas microdontin and aloin showed weak activity against these bacterial strains with MIC values of 400 µg/mL.

All strains of *V. cholerae* employed in this study were susceptible to aloinoside (MIC= 10 µg/mL). These strains are the etiologic agents responsible for cholera, which caused by ingestion of contaminated water and food (Reidl and Klose, 2002; Ubong *et al*., 2011). Cholera episodes are characterized by a sudden onset of massive diarrhea and vomiting (Frank *et al*., 2012). Moreover, *E. coli* which can cause a spectrum of disease ranging from diarrhea to life threatening disease called hemolytic uremic syndrome (HUS) (O'Sullivan *et al*., 2007) was also the most inhibited bacterial pathogens by aloinoside with MIC value of 10 µg/mL. To date, there is no report about antimicrobial activity of microdontin and aloinoside. Therefore the present result suggests the potential of these compounds as antimicrobial agent.

Generally Gram-negative bacteria are more resistant to antimicrobial agents compared with Gram-positive bacteria because they are covered with a phospholipid membrane carrying the structural lipopolysaccharide component that makes their cell wall impermeable to antibacterial substance (Patrone and Stein, 2007). However, the present results revealed that the isolated compounds are much more active against the Gram-negative bacteria than the Gram-positive ones. This activity, therefore, could be explained from the chemical nature of the test samples which might affect the overall impermeability and integrity of the bacterial cell wall.

Literature survey indicates that, anthraquinones isolated from the exudates of *A. vera* possess antibacterial activity (Hamma, 2008). According to Wang *et al*. (2010), the

Table 9: Minimum inhibitory concentrations (MICs) of compounds isolated from the leaf latex of *Aloe sinana* against *some* bacterial strains.

Microorganism	MIC (µg/mL)		
	Microdontin	Aloin	Aloinoside
Bacillus pumilus 82	400	400	NA
B. subtilis ATCC 6633	400	400	NA
Escherichia coli CD/99/1	10	25	10
E. coli K88	10	25	25
E. coli K99	10	25	25
E. coli LT37	10	25	25
E. coli ROW 7/12	10	25	25
E. coli 3:37C	10	25	25
E. coli 306	10	25	25
E. coli 872	10	25	25
Salmonella typhi Ty2	25	25	25
S. enterica TD 01	200	100	50
Shigella boydii D13629	25	25	10
S. dysentery 8	25	25	10
S. flexneri Type 6	25	25	10
S. soneii 1	25	25	10
Staphylococcus aureus ML267	10	50	10
Vibrio cholerae 85	10	25	10
V. cholerae 293	10	25	10
V. cholerae 1313	10	25	10
V. cholerae 1315	10	25	10

NA= No activity.

presence of functional groups such as carboxyl, hydroxyl and hydroxylmethyl on phenyl ring of anthraquinones could improve the antimicrobial activity. These compounds exert their action by increasing membrane permeability of the bacterial cell wall and cause leakage of intracellular contents and lead to cell death (Lu *et al*., 2011; Wu *et al*., 2006). Probable targets in the microbial cell are surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes. Therefore, one possible mechanism of action of the isolated compounds on the employed bacterial strains might be their specific properties on microorganism's cell wall integrity or by some other means yet to be determined.

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in human, pathogenic microorganisms are constantly developing resistance to these drugs (Vaghasiya *et al*., 2011).

The widely spread of resistance to antibiotics is promoting resurgence in the search of antimicrobial agents for the treatment of diseases caused by bacteria (Wendakoon *et al*., 2011). Therefore, the *in vitro* activity of the test samples on the above disease causing bacterial strains is, therefore, highly significant. However, *in vivo* antibacterial and toxicity studies have to be carried out before the compounds are considered for use in the fight against bacterial infection.

4.2.3.3. Antifungal Activity

Comparative evaluation of the isolated compounds showed variation in the level of activity against the four human pathogenic fungal strains tested (Table 10). This variation was evident from their zones of inhibition as well as from their minimum inhibitory concentration values.

Table 10: Diameters of zone of inhibition of the compounds isolated from the leaf latex of *Aloe sinana* at a concentration of 2000 µg/mL against some fungal pathogens.

Fungal strain	Diameter zone of inhibition (mm)			
	Microdontin	Aloin	Aloinoside	Griseofulvin
Aspergillus niger ATCC 6275	13.0(86.7)	12.5(83.3)	11.5(76.7)	15.0
Candida albicans ATCC 10231	14.0(87.5)	13.5(84.4)	12.5(78.1)	16.0
Penicillum funiculosum NCTC 287	13.5(96.4)	12.0(85.7)	11.0(78.6)	14.0
P. notatum ATCC 11625	13.0(96.3)	12.0(88.9)	11.0(81.5)	13.5

Figures in parenthesis indicate % activity of the test sample compared with that of grisofulvin.

P. funiculosum and *P. notatum* were found to be highly susceptible to microdontin, while *A. niger* and *C. albicans* were moderately susceptible to this compound. Aloin showed moderate activity against all the fungal strains tested (MIC= 800 µg/mL). This compound, which was previously isolated from *A. ferox* has been reported to be active against *C. albicans* (Kambizi and Afolayan, 2008). Aloinoside showed moderate activity against *C. albicans,* while *A. niger, P. funiculosum* and *P. notatum* were less sensitive to this compound.

Previous work revealed that anthraquinones isolated from *Rheum emodi* and *Rheum australe* possess antifungal and antibacterial activity against different bacterial and fungal strains (Agarwal *et al*., 2000; Rokaya *et al*., 2012). Moreover, anthraquinone derivatives (rhein, physcion, aloe-emodin and chrysophanol) isolated from *Rheum emodi* showed antifungal activity and the inhibition depends on the presence of substitution such as hydroxyl groups at C-1 and C-8 (Agarwal *et al*., 2000).

Table 11: Minimum inhibitory concentration (MIC) values of compounds isolated from the leaf latex of *Aloe sinana* against some fungal pathogens

Fungal strain	MIC (mM)		
	Microdontin	Aloin	Aloinoside
Aspergillus niger ATCC 6275	800	800	1000
Candida albicans ATCC 10231	800	800	800
Penicillum funiculosum NCTC 287	400	800	1000
P. notatum ATCC 11625	400	800	1000

4.2.4. Antimalarial Activity

4.2.4.1. Microdontin

Microdontin was tested against *P. berghei* (ANKA strain) in mice and showed a dose dependent chemossepressive effect at various doses employed in this study. The chemossupprssion was 71.33%, 63.34%, 52.3%, and 32% at doses 50, 100, 200 and 400 mg/kg/day, respectively (Table 12). The chemossuppression produced by microdontin was statistically significant (P<0.001) when compared to the negative control group. The standard drug (chloroquine) causes 100% chemosuppression at 25 mg/kg/day and this value was significantly (P<0.001) higher than the chemosuppression value obtained by microdontin treated groups.

Mice treated with microdontin had a mean survival time ranging from 8.4 ± 0.51 to 10.2 ± 0.20 days, while the corresponding value for the untreated control group was 7.0 ± 0.001 days. The mice treated with this isolate, except the lower doses (50 and 100 mg/kg/day), survived significantly (P<0.001) longer than mice in the negative

Table 12: Antimalarial activities of microdontin isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei.*

Test substance	Dose mg/kg/day	% Parasitaemia ± SEM	% Suppression	Survival time ± SEM
Vehicle	NC	24.6 ± 0.51	-	7.0 ± 0.001
Microdontin	50	16.66 ± 0.86*	32	8.4 ± 0.51
Microdontin	100	11.7 ± 0.66*	52.3	8.6 ± 0.24
Microdontin	200	8.98 ± 0.26*	63.34	9.4 ± 0.25*
Microdontin	400	7.01 ± 0.35*	71.33	10.2 ± 0.20*
Chloroquine	25	00.00*	100	11.60 ± 0.25

* Mean value is significant (P<0.001) when compared with vehicle treated group; values are presented as mean ± SEM; n=5; NC=negative control.

control group (Table 12). However, although statistically not significant, the mean survival time of mice treated with doses of 50 and 100 mg/kg/day was relatively longer than the negative control. Microdontin showed significant effect on the mean survival time of the treatment groups compared to the untreated control, which increased as the dose increases.

Microdontin not only showed antimalarial activity but also protected the infected mice from weight loss. All microdontin treated groups increased weight when compared to untreated (negative control) groups significantly (P<0.05), except the lower dose (50 mg/kg/day). Mice treated with the higher doses (200 and 400 mg/kg/day) showed better percent weight increment compared to the standard drug chloroquine (Table15). Animals receiving smaller doses of microdontin gained less weight than those treated with higher doses. The highest percent increments (5.31%)

were seen in the group treated with 400 mg/kg/day, while the negative control group showed a decrease in weight (-5.81 %).

Table 13: Body weight of *Plasmodium berghei* infected mice after administration of microdontin.

Test substance	Dose mg/kg/day	Weight D0 ±SEM	Weight D4 ±SEM	% Change
Vehicle	0.5ml	27.68 ± 0.57	26.16 ± 0.44	-5.81
Microdotin	50	26.02 ± 0.28	26.78± 0.25	2.84
Microdotin	100	26.54 ± 0.46	27.24 ± 0.47	2.93*
Microdotin	200	25.84 ± 0.73	27.20 ± 0.60	5.00*
Microdotin	400	26.04 ± 0.47	27.50 ± 0.35	5.31*
Chloroquine	25	26.60 ± 0.43	27.62 ± 0.44	3.69*

Data are expressed as means ± SEM for five mice per group; D0: weight pre-treatment on day zero; D4: weight post-treatment on day five. *the mean value is significant (P<0.05) when compared with vehicle treated group.

4.2.4.2. Aloin

Aloin was tested against *P. berghei* in mice and showed a dose dependent chemossepressive effect at various doses employed in this study. As shown in Table 14, the chemosuppression was 35.3%, 54.3% and 60.1% at doses 100, 200 and 400 mg/kg/day, respectively. The chemosuppression produced by aloin was significant (P<0.001) when compared to the negative control group. The standard drug (chloroquine) caused 100% suppression at 25 mg/kg/day which was higher than the suppression obtained by aloin treated groups.

The mean survival time of aloin treated groups ranged from 7.6 ± 0.25 to 9.4 ± 0.2 days (increased as the dose increases to 400 mg/kg/day). Mice receiving the highest dose of the isolate (400 mg/kg/day) lived longer with mean survival time of 9.4 ± 0.2

Table 14: Antimalarial activity of aloin isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei.*

Test substance	Dose mg/kg/day	% Parasitaemia ± SEM	% Suppression	Survival time ± SEM
Vehicle	NC	26.2 ± 0.37	_	7 ± 0.32
Aloin	100	16.7 ± 0.25*	36.2	7.6 ± 0.25
Aloin	200	11.8 ± 0.25*	55.0	8.4 ± 0.25
Aloin	400	10.3 ± 0.25*	60.1	9.4 ± 0.2
Chloroquine	25	00.00*	100	11.6 ± 0.25*

* Mean value is significant (P<0.001) when compared with vehicle treated group; values are presented as mean ± SEM; n=5; NC=negative control.

days post infection, significantly (P<0.05) beyond the mean survival period of untreated mice. The mean survival time of mice receiving 100 and 200 mg/kg/day was not significant when compared to the negative control. However, although statistically not significant, the mean survival time of mice treated with 100 and 200 mg/kg/day of aloin was relatively higher than the negative control group.

Aloin also protected weight loss of mice infected with *P. berghei.* As shown in Table 15, all aloin treated groups increased weight when compared with the negative control. Mice treated with the highest dose (400 mg/kg/day) of aloin increased weight significantly (P<0.001). Animals receiving lower doses (100 and 200 mg/kg/day) of aloin gained less weight than those treated with the higher doses. Mice receiving the vehicle showed decrease in weight (-8.6) on day four.

Table 15: Body weight of *Plasmodium berghei* infected mice after administration of aloin.

Test substance	Dose mg/kg/day	Weight D0 ±SEM	Weight D4 ±SEM	% Change
Vehicle	0.5 ml	28.67 ± 0.38	26.4 ± 0.43	-8.6
Aloin	100	26.36 ± 0.39	26.8 ± 0.25	1.64
Aloin	200	27.92 ± 0.24	28.5 ± 0.15	2.03
Aloin	400	28.5 ± 0.52	29.6 ± 0.96	3.7*
Chloroquine	25	27 ± 0.22	28.7 ± 0.3	5.9*

Data are expressed as means ± SEM for five mice per group; D0: weight pre-treatment on day zero; D4: weight post-treatment on day five. *the mean value is significant (P<0.001) when compared with vehicle treated group.

4.2.4.3. Aloinoside

Antimalarial suppressive test of aloinoside against *P. berghei* (ANKA strain) in mice showed a dose dependent chemosuppressive effect at various doses employed in the study. The chemosuppression was 53.6%, 65.2% and 85.21% at doses 25, 50 and 100 mg/kg/day, respectively (Table 16). The chemosuppression produced by aloinoside was significant (P<0.001) when compared to the negative control group. The standard drug (chloroquine 25 mg/kg/day) caused 100% suppression and this value was significantly (P<0.001) higher than the chemosuppression value obtained in aloinoside treated groups. In comparison to microdontin or aloin, aloinoside exhibited the highest antimalarial activity.

The mean survival time of mice treated with aloinoside was found to be 9.2 ± 0.49, 9.6 ± 0.25 and 10.6 ± 0.25 days, at doses of 25, 50 and 100 mg/kg/day, respectively (Table 16). The mean survival time were statistically significant (P<0.001) except for the lowest dose 25 mg/kg/day when compared to the untreated mice. Although, statistically not significant, the mean survival time of mice treated with 25 mg/kg/day

Table 16: Antimalarial activity of aloinoside isolated from of *Aloe sinana* in male Swiss albino mice infected with *Plasmodium berghei.*

Test substance	Dose mg/kg/day	% Parasitaemia ± SEM	% Suppression	Survival time ± SEM
Vehicle	NC	23.26 ± 0.88	-	6.8± 0.37
Aloinoside	25	10.8 ± 0.37	53.6*	9.2 ± 0.49
Aloinoside	50	8.1 ± 0.33	65.2*	9.6 ± 0.25*
Aloinoside	100	3.44 ± 0.28	85.21*	10.6 ± 0.25*
Chloroquine	25	00.00	100	11.8 ± 0.2

* Mean value is significant (P<0.001) when compared with the negative control; data are expressed as means ± SEM for five mice per group; NC= negative control.

of aloinoside was relatively longer than the negative control. Aloinoside showed significant effect on the mean survival time of the treatment group compared to the negative group.

In addition to antimalarial activity, aloinoside protected the infected mice from weight loss. All aloinoside treated groups increased weight significantly (P<0.05) when compared to the negative control group. Moreover aloinoside treated groups showed better weight percent increment compared to chloroquine (Table 17). The highest percent increment (7.27%) was seen in the group taking 100 mg/kg/day, while the negative control group showed a decrease in weight (-6.16%).

Table 17: Body weight of *Plasmodium berghei* infected mice after administration of aloinoside.

Test substance	Dose mg/kg/day	Weight D0 ±SEM	Weight D4 ±SEM	% Change
Vehicle	0.5 ml	26.9 ± 0.33	25.34 ± 0.62	-6.16*
Aloinoside	25	26.0 ± 0.35	26.84 ± 0.27	3.13*
Aloinoside	50	25.24 ± 0.54	26.56 ± 0.6	4.97*
Aloinoside	100	24.24 ± 0.55	26.14 ± 0.34	7.27*
Chloroquine	25	26.90 ± 0.33	27.6 ± 0.58	2.54

Data are expressed as means ± SEM for five mice per group; D0: weight pre-treatment on day zero; D4: weight post-treatment on day five. *the mean value is significant (P<0.05) when compared with vehicle treated group.

As shown in the above section, all the isolated compounds possess significant antimalarial activity in 4-day suppression test. Aloinoside recorded the highest chemosupression. The presence of remarkable suppression could be seen also from the life prolonging (prolongation of the mean survival time) potential of the isolated compounds in malaria infected mice. The mice treated with the compounds had significantly longer survival time than the negative control mice. To date, there appears have been no report of aloinoside, microdontin and aloin possessing *in vivo* antiplasmodial activity. The present results therefore suggest that the potential of these compounds as antimalarial agents.

In addition to antiplasmodial activity the isolated compounds also protected the experimental animals from weight loss. Microdontin and aloinoside treated groups show significant weight change even to that of chloroquine treated groups. This effect was also seen in acute toxicity study. The observed largest change in weight might be explained by the fact that these compounds have a direct effect on stimulating appetite. An appetite is regulated by the brain part called hypothalamus. Although, aloin treated

mice not cause weight change like chloroquine, the change is greater than the vehicle treated groups.

Literature survey has revealed that several classes of natural products such as quinoline alkaloids, indoloquinoline alkaloids, carbolines, bis-isoquinoline, 4-quinazole derivatives, trioxanes, terpenes, naphthoquinones, anthraquinones, chalcones, flavanones, coumarins, sesquiterpene lactones and phenolic glycosides possess antimalarial activity (Wube *et al*., 2005). For example, Osman *et al*. (2010) have shown that anthraquinones derivatives isolated from *Rennellia elliptica* Korth (Rubiaceae) possess antiplasmodial activity against chloroquine sensitive strain of *P. falciparum* (3D7) *in vitro*. Moreover, the anthraquinone derivative, aloin has been shown to exhibit *in vitro* antiplasmodial activity against *P. falciparum* (Pillay, 2008).

Overall, the anthraquinone derivatives microdontin, aloin and aloinoside were shown to possess promising antimalarial activity against *P. berghei*. Previous work indicated that hydroxylated anthraquinones such as rufigallol have potent antimalarial activity. Their mode of action was reported to be by acting as a catalytic oxidizing agent capable of undergoing cyclic one-electron oxidation-reduction reactions or as an iron chelator of the parasite-associated iron which is necessary for cellular metabolism and DNA synthesis (Winter *et al*., 1995). As shown in Figure 5, 6 and 7, all the isolated compounds have di-hydroxyl group at C-1 and C-8, which might be by act as an oxidizing agent or as an iron chelator like rufigallol. Moreover, aloinoside, which have two sugars at positions 10 and 11 in addition to the dihydroxyl group was more active than microdontin and aloin suggesting that the sugars could be important for the antimalarial activity, by increasing the solubility of the compound to facilitates its transport to the site of action (García *et al*., 2006).

5. CONCLUSION

In conclusion, the reputed antimicrobial and antimalarial effect of leaf latex of *A. sinana* may be attributed in part or the whole due to the synergetic effects of microdontin, aloin and aloinoside. Moreover, aloinoside could be a promising candidate as an antimalarial compound. The genuine antimicrobial and antiplasmodial activity along with its safety profile observed in the present study could make the leaf latex of *A. sinana* and the isolated compounds to be potential addition to the antimalarial and antimicrobial armamentarium, and also provide scientific support for the ethnomedicinal use of the plant.

6. RECOMMONDATION

Based on the present study the following recommendations are proposed:

1. Investigation of the latex and isolated compounds on other bacterial strains including drug resistant bacterial strains;
2. To perform chronic toxicity studies on latex and isolated compounds;
3. Isolation of minor compounds from the latex of *A. sinana* and study their antimicrobial and antimalarial activities;
4. To perform the possible appetite inducing effect of latex of *A. sinana*, microdontin and aloinoside.

Reference

Abdulelah H.A, Zainal-Abidin BA (2007). *In vivo* antimalarial tests of *Nigella sativa* (Black Seed) different extracts. *American Journal of Pharmacology and Toxicology* **2**: 46-50.

Adebayo, Krettli (2011). Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology* **133**: 289–302.

Adorini L, Arai K, Berek C, Schmitt V, Anne M, Waksman B (2002). Malaria Immunology, 2nd edition, Stockholm, pp 1-2.

Agarwal SK, Singh SS, Verma S, Kumar S (2000). Antifungal activity of anthraquinone derivetives from *Rheum emodi*. *Journal of Ethinopharmacology* **72**: 43-46.

Alshawsh M, Mothana R, Al-shamahy H, Alsllam S, Lindequis U (2009). Assessment of antimalarial activity against *Plasmodium falciparum* and phytochemical screening of some Yemeni medicinal plants *Evidence-Based Complementary and Alternative Medicine* **6**: 453–456.

Amresh, Reddy, Rao, Singh (2007). Evaluation of antiinflammatory activity of *Cissampelos pareira* root in rats. *Journal of Ethnopharmacology* **110**: 526–531.

Arun P, Bhayadiya, Manocha N, Pathan, Dubey (2012). Isolation of aloin from *Aloe Vera*, its characterization and evaluation for antioxidant activity. *International Journal of pharmaceutical reasearch and development* **4**: 024-028.

Asamenew G, Bisrat D, Mazumder A, Asres K (2011). *In vitro* antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana* Reynolds. *Phytotherapy Research* **25**: 1756-1760.

Asres K, Bucar F, Knauder E, Yardley V, Croft S (2001). *In vitro* antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. *Phytotherapy Research* **15**: 613–617.

Assefa A, Urga K, Guta M, Mekonene W, Melaku D, Mudie K, Kidanemariam T (2007). *In vivo* antimalarial activities of plants used in Ethiopian traditional medicine, Delomenna, South East Ethiopia. Ethiopian Health and Nutrition research institute, Ethiopia.

Banzouzi, Prado, Menan, Valentin, Roumestan, Mallié, Pelissier, Blache (2004). Studies on medicinal plants of Ivory Coast: Investigation of *Sida acuta* for *in vitro* antiplasmodial activities and identification of an active constituent. *Phytomedicine* **11**: 338–341.

Bashir A, Saeed B, Mujahid T, Jehan N (2011). Comparative study of antimicrobial activities of *Aloe vera* extracts and antibiotics against isolates from skin infections. *African Journal of Biotechnology* **10**: 3835-3840.

Bekele E (2007). Study on actual situation of medicinal plants in Ethiopia. Japan Association for International Collaboration of Agriculture and Forestry **pp** 20.

Bisrat D, Dagne E, Van Wyk B, Viljoen A (2000). Chromones and anthrones from *Aloe marlothii* and *Aloe rupestris*. *Phytochemistry* **55**: 949-952.

Blitzke T, Porzel A, Masaoud M, Schmidt J (2000). A chlorinated amide and piperidine alkaloids from *Aloe sabaea*. *Phytochemistry* **55**: 979-982.

Blitzke T, Masaoud M, Schmidt J (2001). Constituents of *Aloe rubroviolacea*. *Fitoterapia* **72**: 78-79.

Bloland P (2001). Drug Resistance in Malaria. Geneva: World Health Organization.

Botes L, Van der WF, Loots DT (2008). Phytochemical contents and antioxidant capacities of two *Aloe greatheadii* var. *davyana* extracts. *Molecules* **13**: 2169-2180.

Chen W, Van Wyk B, Vermaak I, Viljoen A (2012). Cape aloes-A review of the phytochemistry, pharmacology and commercialization of *Aloe ferox*. *Phytochemistry Letters* **5**: 1–12.

Clarkson C, Maharaj V, Crouch N, Grace O, Pillay P, Matsabisa M, Bhagwandin N, Smith P, Folb P (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology* **92**: 177–191.

Coopoosamy R, Magwa M (2006). Antibacterial activity of aloe emodin and aloin A isolated from *Aloe excels. African Journal of Biotechnology* **5**: 1092-1094.

Coopoosamy R (2010). Isolation of volatile compounds of *Aloe excels* (Berger). *African Journal of Biotechnology* **9**: 7289-7294.

Coopoosamy R, Magwa M (2007). Traditional use, antibacterial activity and antifungal activity of crude extract of *Aloe excels. African Journal of Biotechnology* **6**: 2406-2410.

Crawley J, Nahlen B (2004). Prevention and treatment of malaria in young African children's. *Seminar in Pediatric infectious disease* **15**: 169-180.

Dagne E (1996). Review of the chemistry of *Aloes* of Africa. *Bulletin of the chemical society of Ethiopia* **10**: 89-103.

Dagne E, Alemu M (1991). Constituents of the leaves of four *Aloe* species from Ethiopia. *Bulletin of the chemical society of Ethiopia* **5**: 87-91.

Dagne E, Yenesew A (1994). Anthraquinones and chemotaxonomy of the asphodelaceae. *Pure and Applied chemistry* **66**: 2395-2398.

Dagne E, Bisrat D, Codina C, Bestda J (1998). A *C, O*-diglucsylated Oxanthrone from *Aloe littoralis*. *Phytochemistry* **48**: 903-905.

Dagne E, Bisrat D, Van Wyk B, Viljoen A, Hellwig V, Steglich W (1997). Anthrones from *Aloe microstigma*. *Phytochemistry* **44**: 1271-1274.

Dagne E, Bisrat D, Viljoen A, Wyk B (2000). Chemistry of *Aloe* Species. *Current organic chemistry* **4**: 1055-1078.

Deharo E, Bourdy G, Quenevo C, Mun˜oz V, Ruiz G, Sauvain M (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology* **77**: 91–98.

Demissew S, Nordal I (2003). Flora of Ethiopia and Eritrea. Aloes and Lilies of Ethiopia and Eritrea, **pp** 42-43.

Deressa T, Mekonnen Y, Animut A (2010). *In vivo* antimalarial activities of *Clerodendrum myricoides, Dodonea angustifolia* and *Aloe debrana* against *Plasmodium berghei. Ethiopian Journal of Health Development* **24**: 25-29.

Department for International Development (DFID) (2011): Malaria: Country Profiles, version 1.1, **pp** 13.

Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W, Melaku D, Assefa A, Makonnen Y (2006). *In vivo* antimalarial activity of hydroalcoholic extracts from *Asparagus africanus* Lam. in mice infected with *Plasmodium berghei*. *Ethiopian Journal of Health and Development* **20**: 112-118.

Elufioye TO, Agbedahunsi JM. (2004). Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febifuga* (Rubiaceae) on mic*e in vivo***.** *Journal of Ethnopharmacology* **93**: 167-171.

Ethiopian National Malaria Indicator Survey (ENMIS) (2011). The Ethiopian Health and Nutrition Research Institute and Partners**, pp** 1.

Fatmawaty, Fadilah, Astuti H (2013). Antimalarial activity of *Delonix regia* on mice with *Plasmodium berghei*. *Journal of Natural Products* **6**: 61-66.

Farah MH, Andersson R, Samuelsson G (1992). Microdontin A and B: two new aloin derivatives from *Aloe microdonta*. *Planta Med*ica **58**: 88-93.

Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S (2004). Antimalarial drug discovery: Efficacy models for compound screening. *Nature* reviews, Drug discovery **3**: 509-520.

Francischetti I, Seydel K, Monteiro R (2008). Blood coagulation, inflammation and malaria. *Microcirculation* **15**: 81–107.

Frank BO, Alfred AD, Alfred S (2012). Cholera and Spatial Epidemiology, Cholera, Dr. Sivakumar Gowder (Editor), ISBN: 978-953-51-0415-5.

Gao J, Zhang G, Dai R, Bi K (2004). Isolation of aloinoside B and metabolism by rat Intestinal bacteria. *Pharmaceutical Biology* **42**: 581–587.

García SK, Villarreal AN, Lübben P, Peña RL (2006). Chrysophanol, antimicrobial anthraquinone from the root extract of *Colubrina greggii*. *Journal of Mexican Chemical Society* **50**: 76-78.

Giday M, Asfaw Z, Elmqvist T, Woldu Z (2003). An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *Journal of Ethnopharmacology* **85**: 43–52.

Giday M, Asfaw Z, Woldu Z (2009). Medicinal plants of the Meinit ethinic group of Ethiopia: An Ethnobotanical study. *Journal of Ethnopharmacology* **124**: 513–521.

Ginsburg H, Deharo E (2011). A call for using natural compounds in the development of new antimalarial treatments-an introduction. *Malaria Journal* **10** (Supp l): S1.

Grace OM, Simmonds MS, Smith GF, Van Wyk A (2010). Chemosystematic evaluation of *Aloe section Pictae* (Asphodelaceae). *Biochemical Systematics and Ecology* **38**: 57–62.

Grimberg BT, Mehlotra RK (2011). Expanding the Antimalarial Drug Arsenal—Now, But How? Review. *Pharmaceuticals* **4**: 681-712.

Grover A, Bhandari BS, Rai N (2011). Antimicrobial Activity of Medicinal plants-*Azadirachta indica* A. Juss, *Allium cepa* L. and *Aloe vera* L. *International Journal of Pharma Tec Reasearch* **3**: 1059-1065.

Hajhashemi V, Ghannadi, Heidari1 AH (2012). Antiinflammatory and wound healing activities of *Aloe littoralis* in rats. *Research in Pharmaceutical Sciences* **7**: 73-78.

Hamman JH (2008). Composition and Applications of *Aloe vera* Leaf Gel. *Molecules* **13**: 1599-1616.

Health Sector Development Programme IV (HSDP) (2010). Federal Democratic Republic of Ethiopia Ministry of Health, Ethiopia.

Hecht DW, Citron DM, Cox M, Jacobus N, Jenkins SG, Onderdonk A, Roe-Carpenter D, Rosenbatt JE, Wexler HM (2006). Method for antimicrobial susceptibility testing of anaerobic bacteria; approved standard-seventh edition. *Clinical and Laboratory Standard Institute* **27**: ISBN1-56238-626-3.

Holzapfel CW, Wessels PL, VanWyk BE, Marais W, Portwig M (1997). Chromone and aloin derivatives from *Aloe broomii A. Africana* and *A. speciosa. Phytochemistry* **45**: 97-102.

James DA, Harrison TS (2012). Immunotherapy for fungal infections. *Current Opinion in Microbiology* **15**: 434–439.

Jayasinghe CD, Udagama-Randeniya PV, Ratnasooriya WD (2008). *In vivo* antimalarial activity of aqueous root extract of *Barringtonia acutangula* in mice. *Pharmacognosy Magazine* **4**: 51-58.

Kalra BS, Chawla S, Gupta P, Valecha N (2006). Screening of antimalarial drugs. *Indian Journal of Pharmacology* **38**: 5-12.

Kambizi L, Afolayan AJ (2008). Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhea. African Journal of Biotechnology* **7**: 012-015.

Kambizi L, Sultana N, Afolayan AJ (2004). Bioactive Compounds isolated from *Aloe ferox:* A plant traditionally used for the treatment of sexually transmitted infections in the Eastern Cape, South Africa. *Pharmaceutical Biology* **42**: 636-639.

Kasa M, Mekonnen Y, W/Micheal T, Mohamed H, Balcha S (2005). Therapeutic efficacy of mefloquine and sulfadoxine/pyrimethamine for the treatment of uncomplicated plasmodium falciparum malaria in children, Metehara town, Southeast, Ethiopia. *Ethiopian Journal of Health Development* **19**: 167-163.

Kassaye KD, Amberbir A, Getachew B, Mussema Y (2006). A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiopian Journal of Health Development.* **20**: 127-134.

Katzung B (2006). Basic and clinical Pharmacology. In Chemotherapeutic Drugs, 10th edition, Katzung BG (editor), Mc Graw Hill Lange, San Francisco, USA, **pp**-53.

Khanna N (2007). Pharmacology, Antimicrobial Agents: Antiprotozoal Drugs. Department of Pharmacology, University College of Medical Sciences, Shahdara, Delhi, New York.

Klotsas E, Lever A (2007). An update on malaria prevention, diagnosis and treatment for the returning traveler. *Blood Review* **21**: 73–87.

Kullberg B (1997).Trends in immunotherapy of fungal infections. *Current Tropic: Fungal Infections* **16**: 51-55.

Kuzuya H, Tamai I, Beppu H, Shimpo K, Chihara T (2001). Determination of aloenin, barbaloin and isobarbaloin in *Aloe* species by micellar electrokinetic chromatography. *Journal of Chromatography* **752**: 91–97.

Lu C, Wang H, Lv W, Xu P, Zhu J, Xie J, Liu B, Lou Z (2011). Antibacterial properties of anthraquinones extracted from rhubarb against *Aeromonas hydrophila*. *Fisheries Science* **77**: 375–384.

Lundqvist J (2009). Malaria and relapsing fever *Borrelia* interaction and potential therapy. Msc. thesis, UMEA University, Faculty of Medicine, Departement of Molecular Bology.

Magwa M, Gundidza M, Coopoosamy R, Mayekiso B (2006).Chemical composition of volatile constituents from the leaves of *Aloe ferox*. *African Journal of Biotechnology* **5**: 1652-1654.

Mehrotra S, Ashwani, Srivastava, Nandi S (2010). Comparative antimicrobial activities of Neem, Amla, *Aloe*, Assam Tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa. Journal of Medicinal Plants Research* **4**: 2473-2478.

Mesfin A, Giday M, Animut A, T/Haymanot T (2011). Ethnobotanical study of antimalarial plants in Shinile District, Somali Region, Ethiopia, and *in vivo* evaluation of selected ones against *Plasmodium berghei. Journal of Ethnopharmacology* **139**: 221–227.

Metowogo K, Agbonon A, Eklu GK, Aklikokou, Gbeassor AK (2008). Anti-ulcer and Anti-inflammatory Effects of Hydroalcohol Extract of *Aloe buettneri* A. Berger (Lilliaceae). *Tropical Journal of Pharmaceutical Research* **7**: 907-912.

Mitchell JK, Carter WE (2000). Modeling antimicrobial activity of Clorox^{TM} using an agar-diffusion test: A new twist on an old experiment. *Bioscience* **26**: 9-13.

Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi EN (2007). Traditional phytotherapy of some remedies used in treatment of malaria in Meru district of Kenya. *South African Journal of Botany* **73**: 402–411.

Mwale M, Masika PJ (2010). Analgesic and anti-inflammatory activities of *Aloe ferox Mill.* aqueous extract. *African Journal of Pharmacy and Pharmacology* **4**: 291-297.

Nandal U, Bhardwaj RL (2012). *Aloe vera* for human nutrition, health and cosmetic use - A review. *International Research Journal of Plant Science* **3**: 038-046.

NEHC (2000). Navy Environmental Health Center Technical Manual, Bureau of Medivine and Surgery.

Ndhlala AR, Amoo SO, Stafford GI, Finnie JF, Staden JV (2009). Antimicrobial, anti-inflammatory and mutagenic investigation of the South African tree aloe (*Aloe barberae*). *Journal of Ethnopharmacology* **124**: 404–408.

National Institute of Allergy and Infectious Diseases (NIAD) (2007). Understanding malaria, U.S. Department of Health and Human services.

OECD guideline for testing of chemicals (2008). Acute Oral Toxicity: Acute Toxic Class Method.

Okyere KA, Asante FA, Tarekegn J, Andam KS (2011). A review of the economic impact of malaria in agricultural development. *Agricultural Economics* **42**: 293–304.

Ongkana R (2003). Phytochemistry and antimalarial activity of *Eupatorium odoratum* L. Msc. Thesis, Departement of Pharmaceutical chemistry and phytochemistry. Mahidol University, Thiland.

Onyeibor O, Croft SL, Dodson HI, Feiz Haddad M, Kendrick H, Millington NJ, Parapini S, Phillips RM, Seville S, Shnyder SD, Taramelli D, Wright CW (2005). Synthesis of some cryptolepine analogues, assessment of their antimalarial and cytotoxic activities, and consideration of their antimalarial mode of action. *Journal of Medicinal Chemistry* **48**: 2701-2709.

Osman CP, Ismail NH, Ahmad R, Ahmat N, Awang K, Jaafar FM (2010). Anthraquinones with antiplasmodial activity from the roots of *Rennellia elliptica* Korth. (Rubiaceae). *Molecule* **15**: 7218-7226.

O'Sullivan J, Bolton DJ, Duffy G, Baylis C, Tozzoli R, Wasteson Y, Lofdahl S (2007). Methods for detection and molecular characterization of pathogenic *Escherichia coli*. *Pathogenic Escherichia coli Network* ISBN 1-84170-506-3.

Patrone JB, Stein DC (2007). Effect of gonococcal lipooligosaccharide variation on human monocytic cytokine profile. *Bio Med Central Microbiology* **7**: 1-15.

Pillay A (2008). Synthesis and biological activities of aloin derivetives. MSc Thesis, School of Chemistry, University of Kwazulu-Natal, Pietermaritzburg.

Reidl J, Klose KE (2002). *Vibrio cholerae* and cholera: out of the water and into the host. *Federation of European Microbiological Societies Reviews* **26**: 125-139.

Rokaya MB, Munzbergova Z, Timsina B, Bhattarai KR (2012). *Rheum australe* D. Don: A review of its botany, ethnobotany, phytochemistry and pharmacology. *Journal of Ethinopharmacology* **141**: 761-774.

Reynolds T (2005). Hemlock alkaloids from Socrates to poison *Aloes*. *Phytochemistry* **66**: 1399–1406.

Sanmukhiya R, Soulange G, Christophe L, Khoyratty S, Silva D, Frederich M, Kodja H (2010). Molecular biology, phytochemistry and bioactivity of three endemic *Aloe* Species from Mauritius and Réunion Islands. *Phytochemical Analysis* **21**: 566-574.

Sanon S, Azas N, Gasquet M, Ollivier E, Mahiou V, Barro N, Cuzin-Ouattara N, Traore AS, Esposito F, Balansard G, Timon-Davia P (2003). Antiplasmodial activity of alkaloid extracts from *Pavetta crassipes* (K. Schum) and *Acanthospermum hispidum* (DC), two plants used in traditional medicine in Burkina Faso. *Parasitology Research* **90**: 314–317.

Scheibel L (1997). Modern Pharmacolgy with Clinical Applications, In Antiprotozoal Drugs, 5th edition, **pp** 613-614.

Schlitzer M (2008). Antimalarial Drugs – What is in Use and what is in the Pipeline? Arch. Pharm. Chem. Life Science. **341**: 149–163.

Shapiro T, Glodberg D (2006). Good Man & Gilman's the Pharmacological basis of Therapeutics. In chemotherapy of protozoal infections: Malaria, 11th edition, McGraw-Hill (editors), New York Chicago San, Chapter 39.

Shargie EB, Gebre T, Ngondi J, Graves P, Mosher AW, Emerson PM, Ejigsemahu Y, Endeshaw T, Olana D, W/Meskel A, Teferra A, Tadesse Z, Tilahun A, Yohannes G, Richards FO (2008). Malaria prevalence and mosquito net coverage in Oromia and SNNPR regions of Ethiopia. *Bio Med Central Public Health* **8**: 321.

Sonia M, Mohamed D (2008). *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. *Journal de la Société Chimique de Tunisie*. **10**: 101-109.

Stangelanda T, Aleleb PE, Katuura E, Lyea KA (2011). Plants used to treat malaria in Nyakayojo sub - county, western Uganda. *Journal of Ethnopharmacology* **137**: 154–166.

Talisuna AO, Bloland P, Alessandro UD (2004). History, dynamics and public health importance of malaria parasite resistant. *Clinical Microbiology Review* **17**: 235-254.

Ubong, A, Tunung, R, Noorlis, A, Elexson, N, Zainazor T, Ghazali, FM, Nakaguchi, Nishibuch, M, Son, R (2011). Prevalence and detection of *Vibrio* spp. and *Vibrio cholerae* in fruit juices and flavored drinks. *International Food Research Journal* **18**: 1163-1169.

University of California Davis Botanical Conservatory (UCDAVIS) (2009). Botanical notes.

United Nations Childeren's Fund (Unicef) (2010). Malaria in Ethiopia, Addis Ababa, Ethiopia **pp** 3-4.

Vaghasiya Y, Patel H, Chanda S (2011). Antibacterial activity of *Mangifera indica* L. seeds against some human pathogenic bacterial strains. *African Journal of Biotechnology* **10**: 15788-15794.

Van Heerdem FR, Van Wyk BE, Viljoen AM (1996). Aloeresin E and F, two chromone derivatives from *Aloe Peglereae*. *Phytochemistry* **43**: 867-869.

Van Heerden FR, Viljoen AM, Van Wyk BE (2000). 6'- *O*- Coumaroyl aloesin from *Aloe castanea*-a taxonomic marker for *Aloe* section Anguialoe. *Phytochemistry* **55**: 117-120.

Vilijoen AM, Van Wyk BE, Van Heerden FR (1998). Distribution and chemotaxonomic significance of flavonoids in *Aloe* (Asphodelaceae). *Plant Systematics and Evolution* **211**: 31-42.

Viljoen AM, Van Wyk BE, Newton L (2001). The occurrence and taxonomic distributions of the anthrones aloin, aloinoside and microdontin in *Aloe*. *Biochemical Systematics and Ecology* **29**: 53-67.

Viljoen AM, Van Wyk BE (2000). The chemotaxonomic significance of the phenyl pyrone aloenin in the genus *Aloe*. *Biochemical Systematics and Ecology* **28**: 1009-1017.

Viljoen A, Van Wyk BE, Van Heerden FR (2002). The chemotaxonomic value of the diglucoside anthrone homonataloside B in the genus *Aloe*. *Biochemical Systematics and Ecology* **30**: 35–43.

Volfson C, Gutterman Y (1998). Content and distribution of anthrone C-glycosides in the South African arid plant species *Aloe mutabilis* growing in direct sunlight and in shade in the Negev Desert of Israel. *Journal of Arid Environments* **40**: 441-451.

Waako PJ, Gumede B, Smith P, Folb PI (2005). The *in vitro* and *in vivo* antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. *Journal of Ethnopharmacology* **99**: 137–143.

Wamer WG, Vath P, Falvey DE (2003). *In vitro* studies on the photobiological properties of aloe emodin and aloin A. *Free Radical Biology & Medicine* **34**: 233–242.

Wang J, Zhao H, Kong W, Jin C, Zhao Y, Qu Y, Xiao X (2010). Microcalorimetric assay on the antimicrobial property of five hydroxyanthraquinone derivetives in rhubarb (*Rheum palmatum L*) to *Bifidobacterium adolescentis*. *Phytomedicine* **17**: 684-689.

Wendakoon C, Calderon P, Gagnon D (2011). Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *Journal of Medicinally Active Plants* **1**: 60-68.

Wessels P, Holizapfel C, Van Wyk BE, Marais W (1996). Plictoside, an *O*, *O*-diglucosylated naphthalene derivative from *Aloe Plicatilis*. *Phytochemistry* **41**: 1547-1551.

Winter RW, Cornell KA, Johnson LL, Isabelle LM, Hinrichs DJ, Riscoe MK (1995). Hydroxy-anthraquinones as antimalarial agents. *Bioorganic and Medicinal Chemistry letters* **5**: 1927-1932.

World Health Organization (WHO) (2011). World Malaria Report 2011, Jeneva, Switzerland.

Wu YW, Ouyang J, Xiao XH, Gao WY, Liu Y (2006). Antimicrobial Properties and Toxicity of Anthraquinones by Microcalorimetric Bioassay. *Chinese Journal of Chemistry* **24**: 45-50.

Wube A, Bucar F, Asres K, Gibbons S, Rattray L, Croft S (2005). Antimalarial Compounds from *Kniphofia foliosa* Roots. *Phytotherapy Research* **19**: 472–476.

Wube A, Bucar F, Asres K, Gibbons S, Rattray L, Croft S (2010). Antiprotozoal Activity of Drimane and Coloratane Sesquiterpenes towards *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum in vitro. Phytotherapy Research* **24**: 1468–1472.

Yagi A, Makino K, Nishioka I (1973). Studies on the Constituent of *Aloe saponaria*. The structure of tetrahydroanthracene derivetives and related anthraquinones. *Chemical and Pharmaceutical Bulletin* **22**: 1159-1166.

Yagi A, Makino K, Nishioka I (1977).Studies on the Constituents of *Aloe saponaria*. The Structures of Phenol Glucosides. *Chemical and Pharmaceutical Bulletin* **25**: 1771-1776.

Yang QY, Yao CS, Fang WS (2010). A new triglucosylated naphthalene glycoside from *Aloe vera*. *Fitoterapia* **81**: 59–62.

Yineger H, Kelbessa E, Bekele T, Lulekal E (2008). Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. *Journal of Medicinal Plants Research* **2**: 132-153.

Appendix 1: List of Antimalarial Ethnomedicinal Plants (Mesfin *et al*., 2011; Stangelanda *et al*., 2011; Muthaura *et al*., 2007;Alshawash *et al*., 2009; Waako *et al*., 2005;Sanon *et al*., 2003).

	Scientific name	**Family**
1	*Acalypha fruticosa L.*	Euphorbiaceae
2	*Acalypha indica L.*	Euphorbiaceae
3	*Acanthospermum hispidum (DC)*	Asteraceae
4	*Acokanthra schimperi Schweeinf.*	Apocynaceae
5	*Ajuga remota Benth*	Labiatae
6	*Albizia gummifera*	Mimosaceae
7	*Aloe*	Aloaceae
8	*Aloe kedongensis*	Aloeaceae
9	*Aloe secundiflora*	Aloeaceae
10	*Anethum graveolens L.*	Apiaceae
11	*Arachis hypogea*	Fabaceae
12	*Aristolochia elegans Mast*	Aristolochiaceae
13	*Artemisia annua*	Asteraceae
14	*Asparagus africanus Lam.*	Asparagaceae
15	*Azadirachta indica A.*	Meliaceae
16	*Azadirachta indica A. Juss*	Meliaceae
17	*Azadirachta indica A.Juss*	Meliaceae
18	*Balanitis rotundifolia*	Balanitaceae
19	*Boscia angustifolia A.*	Rubiaceae
20	*Boswellia elongata Balf. f.*	Burseraceae
21	*Caesalpinia volkensii Harm*	Caesalpinaceae
22	*Cardiospermum halicacabum L.,*	Sapindaceae
23	*Carica papaya L.*	Caricaceae
24	*Carissa edulis Forssk*	Apocynaceae
25	*Carissa spinarum Lodd. ex*	Apocynaceae
26	*Cassia didymobotrya*	Leguminosae
27	*Cissampelos pareira L*	Menispermaceae
28	*Cissus rotundifolia (Forssk). Vahl*	Vitaceae
29	*Cissus rotundifolia (Forssk.)*	Vitaceae
30	*Clausena anista (Willd.)*	Rutaceae
31	*Clematis brachiata Thunb*	Ranunculaceae
32	*Clutia abyssinica Jaub. & Spach*	Euphorbiaceae
33	*Clutia abyssinica Jaub. &Spach*	Euphorbiaceae
34	*Conyza pyrrhopappa Sch.Bip.*	Asteraceae
35	*Cronquist*	Conyza bonariensis (L.)
36	*Cucumis ficifolius A. Rich.*	Cucurbitaceae
37	*Cymbopogon citratus Stapf*	Poaceae
38	*Cyperus articulatus L.*	Cyperaceae
39	*Dendrosicyos socotrana Balf. f.*	Cucurbitaceae

40	*Echium vulgare L.*	Echium vulgare L.
41	*Fagaropsis angolensis*	Rutaceae
42	*Foeniculum vulgare Miller*	Apiaceae
43	*Fuerstia africana*	Lamiaceae
44	*Halothamnus somalensis Botsch.*	Chenopodiaceae
45	*Harrisonia abyssinica*	Simaroubaceae
46	*Hoslundia opposita Vahl*	Lamiaceae
47	*Hydnora johannis Becc.*	Hydnoraceae
48	*Indigofera articulata Gouan*	Fabaceae
49	*Indigofera coerulea Roxb.*	Fabaceae
50	*Jatropha curcas L.*	Euphorbiaceae
52	*Justicia betonica*	Acanthaceae
53	*Kigelia africana (Lam.)*	Bignoniaceae
54	*Lantana trifolia L.*	Verbenaceae
55	*Lawsonia inermis L.*	Lythraceae
56	*Leptadenia hastata (Pers.) Decne.*	Asclepiadaceae
57	*Maerua oblongifolia (Forssk.) A. Rich.*	Capparidaceae
58	*Maesa lanceolata Forssk*	Myrsinaceae
59	*Markhamia lutea (Benth.) K. Schum.*	Bignoniaceae
60	*Maytenus arbutifolia*	Celastraceae
61	*Maytenus heterophylla*	Celastraceae
62	*Maytenus putterlickioides*	Celastraceae
63	*Mentha spicata L.*	Lamiaceae
64	*Momordica foetida Schumach*	Cucurbitaceae
65	*Momordica foetida Schumch. EtThonn*	Cucurbitaceae
66	*Musa paradisiaca*	Musaceae
67	*Myrica kandtiana Engl*	Myricaceae
68	*Myrica salicifolia A.Rich.*	Myricacea
69	*Neoboutonia macrocalyx*	Euphorbiaceae
70	*Ocimum spicatum Deflers*	Lamiaceae
71	*Ocotea usambarensis*	Lauraceae
72	*Olea europaea L*	Oleaceae
73	*Passiflora edulis Sims*	Passifloraceae
74	*Pavetta crassipes*	Rubiaceae
75	*Pentas longiflora Oliv.*	Rubiaceae
76	*Periploca linearifoli*	Asclepiadaceae
77	*Physalis peruviana Mill*	Solanacaea
78	*Pittosporum viridiflorum*	Pittosporaceae
79	*Plectranthus sp.*	Plectranthus cf. forskohlii
80	*Prunus africana (Hook.f.)*	Rosaceae
81	*Pupalia micrantha Hauman*	Amaranthaceae
82	*Rhamnas staddo A. Rich*	Rhamnaceae
83	*Rhamnus prinoides L.*	Rhamnaceae

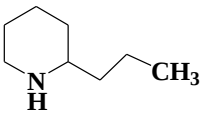
84	*Salvadora persica L.*	Salvadoraceae
85	*Schkuhria pinnata*	Compositae
86	*Senna italica Mill.*	Fabaceae
87	*Solanum incanum L.*	Solanaceae
88	*Solanum sp.*	Solanaceae
89	*Sphaeranthus suaveolens*	Compositae
90	*Strychnos henningsii*	Loaniaceae
91	*Tamarindus indica L.*	Fabaceae
92	*Toddalia asiatica (L.)*	Rutaceae
93	*Toddalia asiatica Lam.*	Rutaceae
94	*Trimeria grandifolia ssp. tropica*	Salicaceae
95	*Vernonia auriculifera*	Compositae
96	*Vernonia brachycalyx O.*	Compositae
97	*Vernonia lasiopus O.*	Compositae
98	*Withania somnifera (L.)*	Solanaceae
99	*Withania somnifera (L.) Dunal*	Solanaceae
100	*Zanthoxylum usambarense*	Rutaceae
101	*Zanthroxylum chalybeum*	Rutaceae

Appendix 2: Compounds and their structures isolated from leaves of *Aloe.*

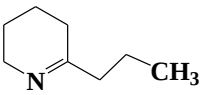
Class of compounds	Structure No	Source	Reference
Alkaloides			
coniine	**6**	*A. sabaea*	Blitzke *et al*., 2000
γ-coniceine	**7**	*A. sabaea, A. chabaudii, A. globuligemma A. ortholopha*	Blitzke *et al*., 2000; Reynolds, 2005
N, N- dimethylconiine	**8**	*A. sabaea*	Blitzke *et al*., 2000
Anthraquinonnones and Pre-Antraquinones			
aloe-emodin-11-O-rhamnoside	**9**	*Aloe spp*.	Dagene *et al*., 2000
nataloe-emodin-2-O-glucoside	**10**	*Aloe spp.*	Dagene *et al*., 2000
aloe-emodin	**1**	*A. ferox; A. excels*	Dagene *et al*., 2000; Kambizi *et al*. (2004): Coopoosamy and Magwa, 2006
crysophanol	**2**	*A. ferox*	Kambizi *et al*. (2004)
aloesaponarin I	**11**	*A. saponaria*	Yagi, 1973
aloesaponarin II	**12**	*A. saponaria*	Yagi, 1973
desoxyerythrolaccin	**13**	*A. saponaria*	Yagi, 1973
laccaic acid D methyl ester	**14**	*A. saponaria*	Yagi, 1973
Anthrones			
aloin A / B	**3**	*A.marlothii A. ferox: A.excels*	Pillay, 2008; Coopoosamy and Magwa, 2006.
aloinoside A / B	**15**	Cape *Aloes A. africana*	GaoJun *et al*., 2004; Holzapfel *et al*., 1997).
homonataloin	**5**	*A. mutabilis*	Volfson and Gutterman, 1998; Pillay, 2008
nataloin	**16**	*A. mutabilis*	Volfson and Gutterman, 1998;
homonataloside	**17**	*Aloe Spp.*	Viljoen *et al*., 2002
microstigmin A	**18**	*A. microstigma*	Dagne *et al*., 1997
5-Hydroxyaloin A	**19**	*A. microstigma*	Dagne *et al*., 1997

5-hydroxyaloin A 6'-*O*-acetate	**20**	*A. marlothii*	Bisrat *et al*., 2000
Microdontin A/B	**21**	*A.microdonta*	Farah *et al*., 1992
Littoraloin	**22**	*A. littoralis*	Dagne *et al*., 1998
deacetyllittoraloin	**23**	*A. littoralis*	Dagne *et al*., 1998
10-hydroxyaloin B	**24**	*A. littoralis*	Dagne *et al*., 1998
Benzene and Naphtalene Derivatives			
aloveroside A	**25**	*A. vera*	Yang *et al*., 2010
plicataloside	**26**	*A. plictailis*	Wessels *et al*., 1996
isoeleutherol	**27**	*A. saponaria*	Yagi *et al*., 1977
Chromones			
aloesin	**28**	*Aloe spp.*	Chen *et al*., 2012
aloeresin A	**29**	*Aloe spp.*	Dagne *et al*., 2000
aloeresin E	**30**	*A. rubroviolacea*	Blitzke *et al*., 2001
aloeresin F	**31**	*A. peglerae*	Van *et al*.(1996)
7-O- methylaloeresin A	**4**	*A. rupestris*	Bisrat *et al*., 2000
Flavonoids			
naringenin	**32**	*Aloe spp.*	Vilijoen *et al*., 1998
dihydroisorhamnetin	**33**	*Aloe spp*	Vilijoen *et al*., 1998
apigenin	**34**	*Aloe spp*	Vilijoen *et al*., 1998
isovitexin	**35**	*Aloe spp*	Vilijoen *et al*., 1998
isoorientin	**36**	*A. parvibracteata*	Grace *et al*., 2010
Coumarins			
Aloenin	**37**	*A. arborescens*	Viljoen and Wyk, 2000; Kuzuya *et al*., 2001
6'-O-coumaroylaloesin	**38**	*A. castanea*	Van *et al*., 2000
Sterols			
cholesterol	**39**	*A. rubroviolacea*	

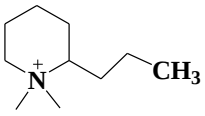
24-methylcholesta-5, 22-dien-3β-ol	**40**	*A. rubroviolacea*	Blitzke *et al*., 2001
campesterol	**41**	*A. rubroviolacea*	
campestanol	**42**	*A. rubroviolacea*	
stigmasterol	**43**	*A. rubroviolacea*	
sitosterol	**44**	*A. rubroviolacea*	
sitostanol	**45**	*A. rubroviolacea*	
β-sitosterol	**46**	*A. greatheadii*	Botes *et al*., 2008
Volatile Constituents			
limonene	**47**	*A. excels*	Coopoosamy, 2010
carvone	**48**	*A. excels*	
phenylacetonitrile	**49**	*A. excels*	
3, 6 octatriene	**50**	*A. ferox*	
3-cyclohexane-1-hetanol	**51**	*A. ferox*	Magwa M *et al*., 2006
Bornylene	**52**	*A. ferox*	
1, 3-cyclopentadiene	**53**	*A. ferox*	
5- methyl- 3-heptanol	**54**	*A. ferox*	



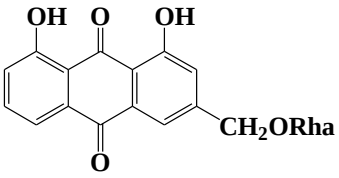
6



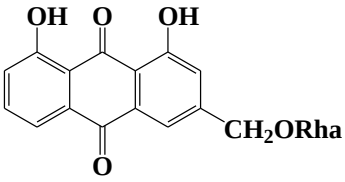
7



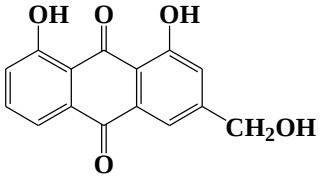
8



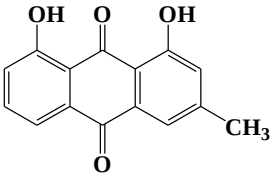
9



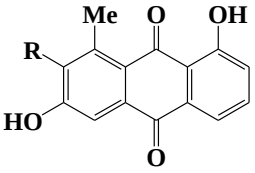
10



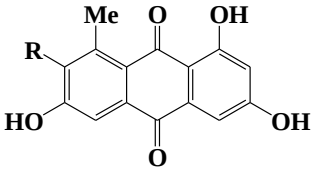
1



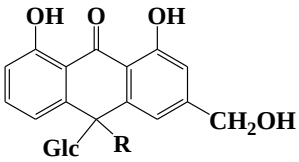
2



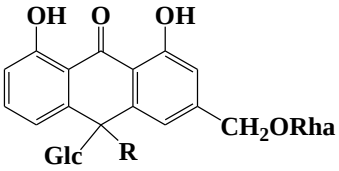
11:R=COOMe
12:R=H



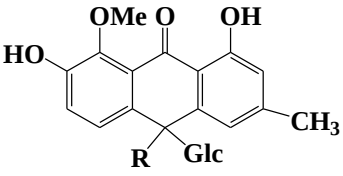
13:R=H
14:R=COOMe



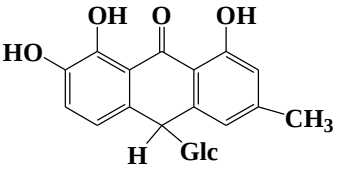
3a, R= **H**
3b, R= **H**



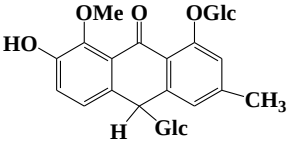
15a, R= **H**
15b, R= **H**



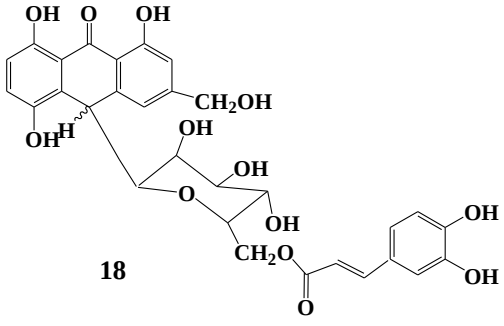
5a,R= **H**
5b,R= **H**



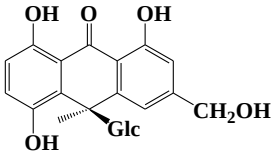
16



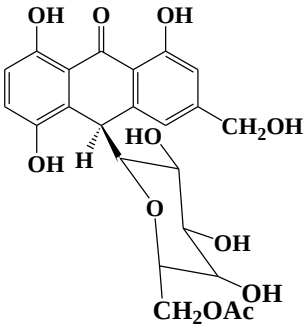
17



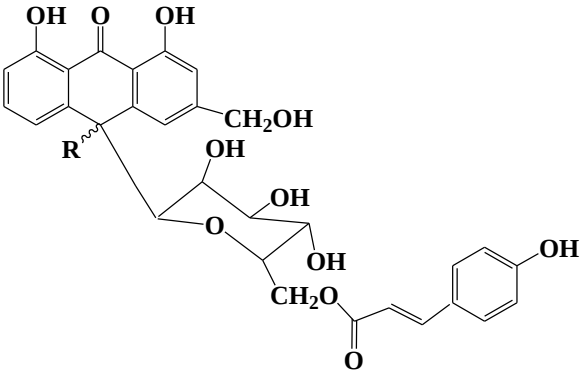
18



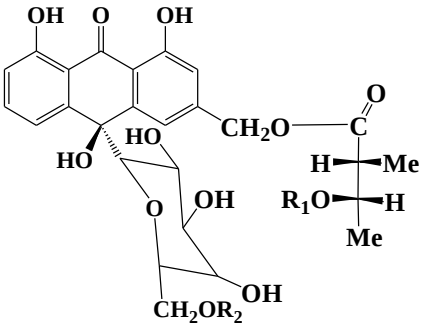
19



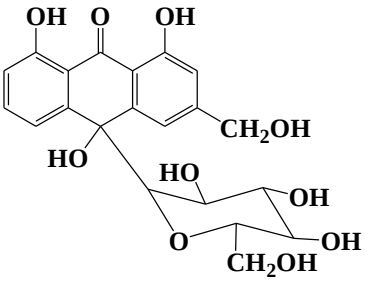
20



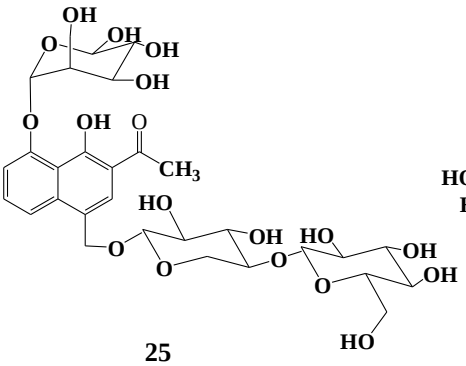
21a, R= **H**
21b, R= **H**



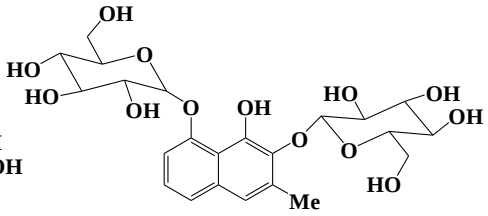
22,R_1=H, R_2=Ac
23,R_1=H, R_2=H



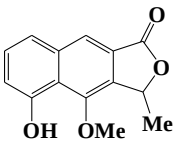
24



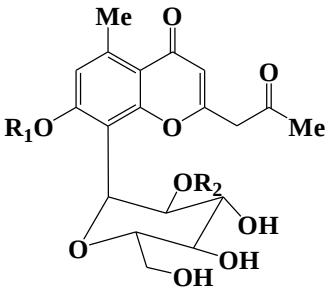
25



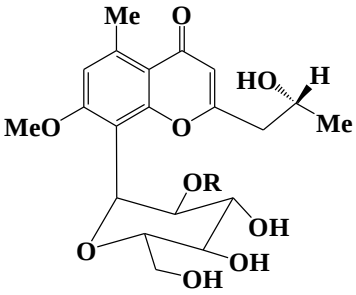
26



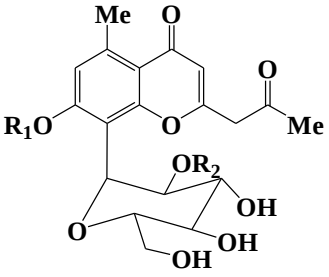
27



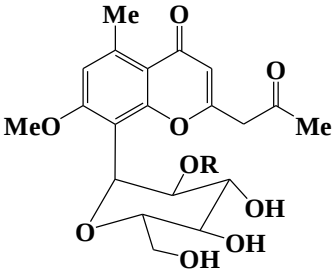
28, R_1=H; R_2=H
29, R_1=H; R_2=p-coumaroyl



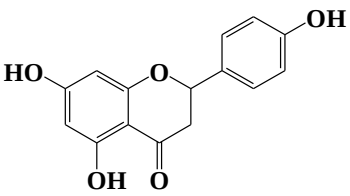
30, R= cinnamoyl



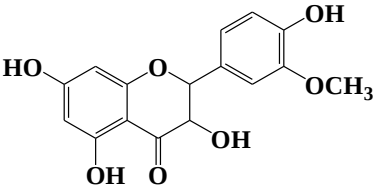
31, R_1=H; R_2= cinnamoyl



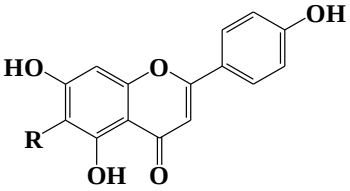
4, R= coumaroyl



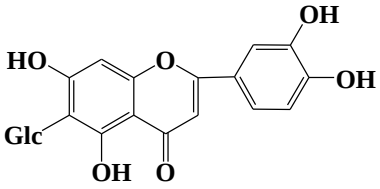
32



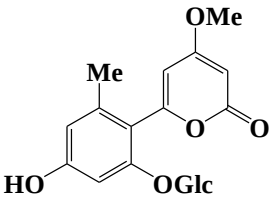
33



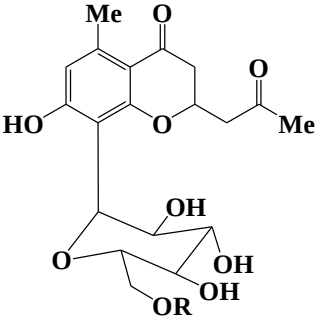
34, R=H
35, R=Glc



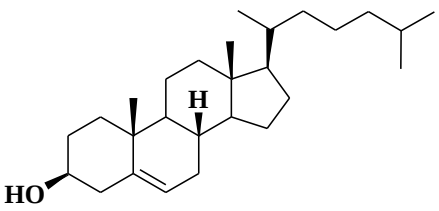
36



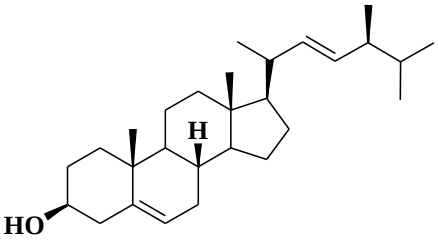
37



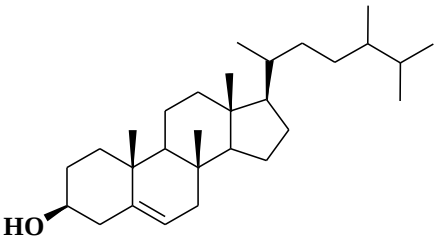
38



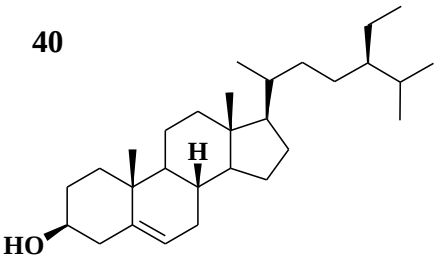
39



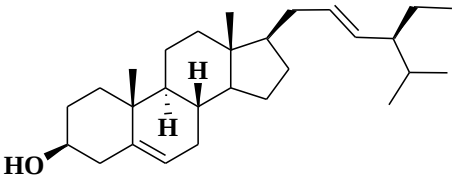
40



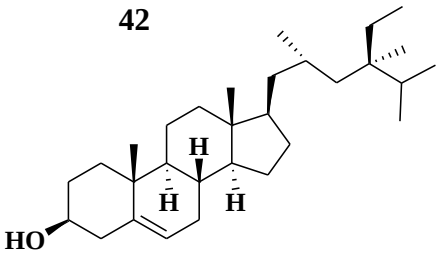
41



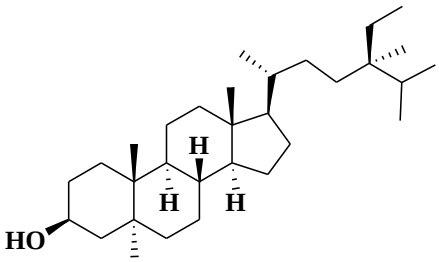
42



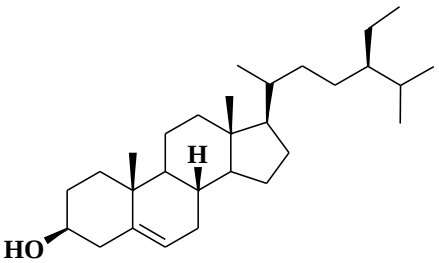
43



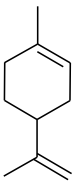
44



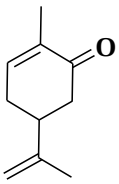
45



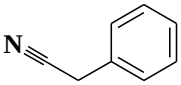
46



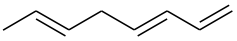
47



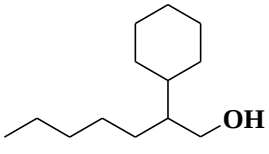
48



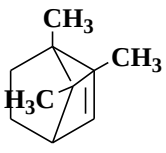
49



50

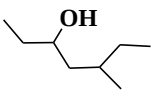


51



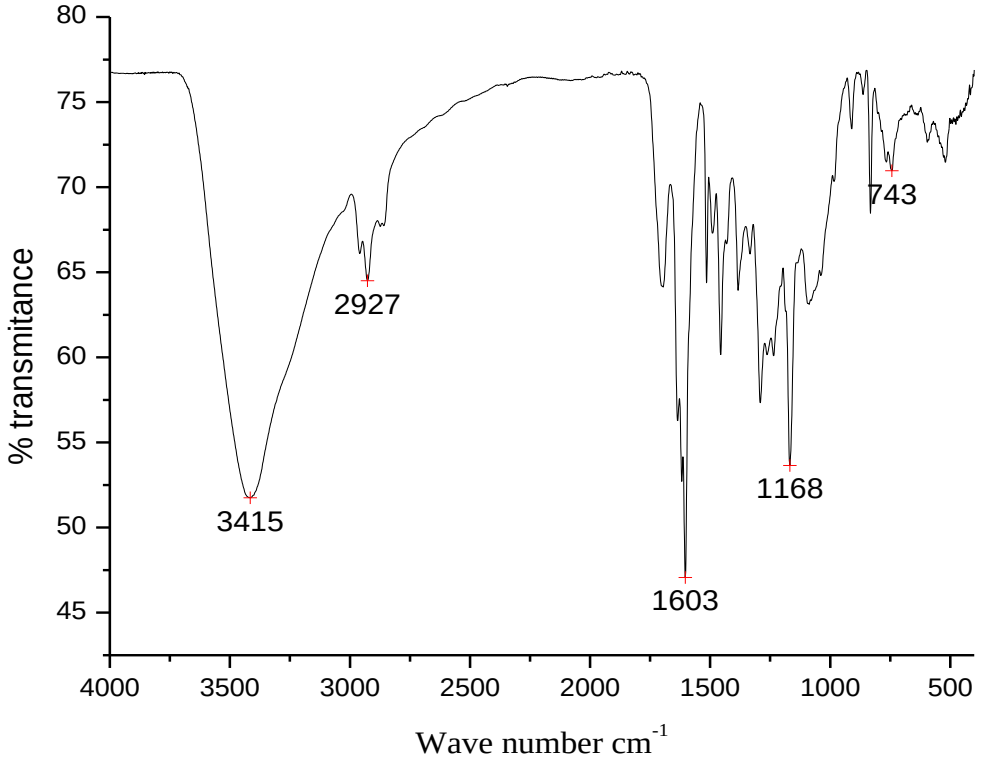
52

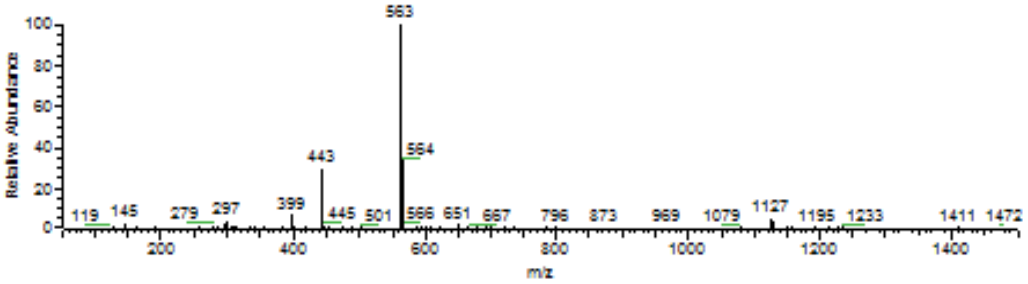
53

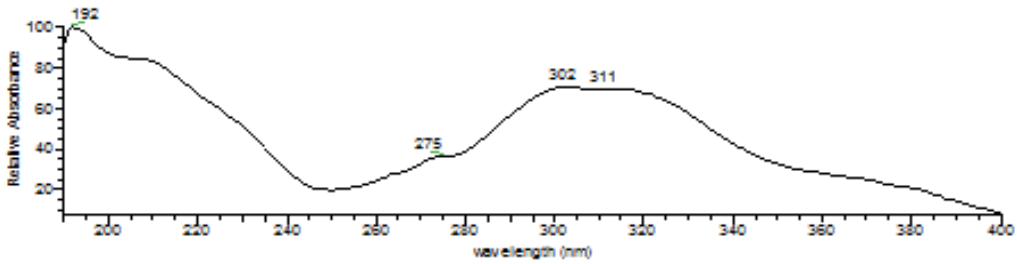


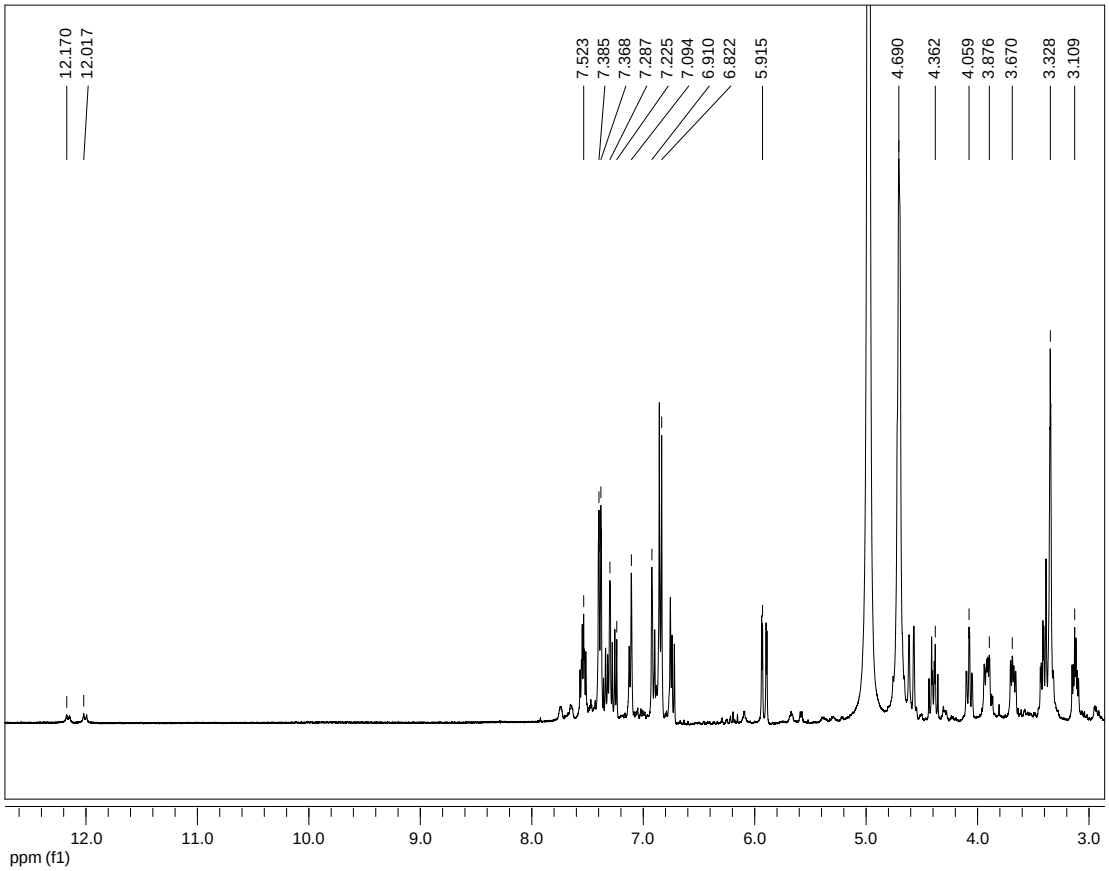
54

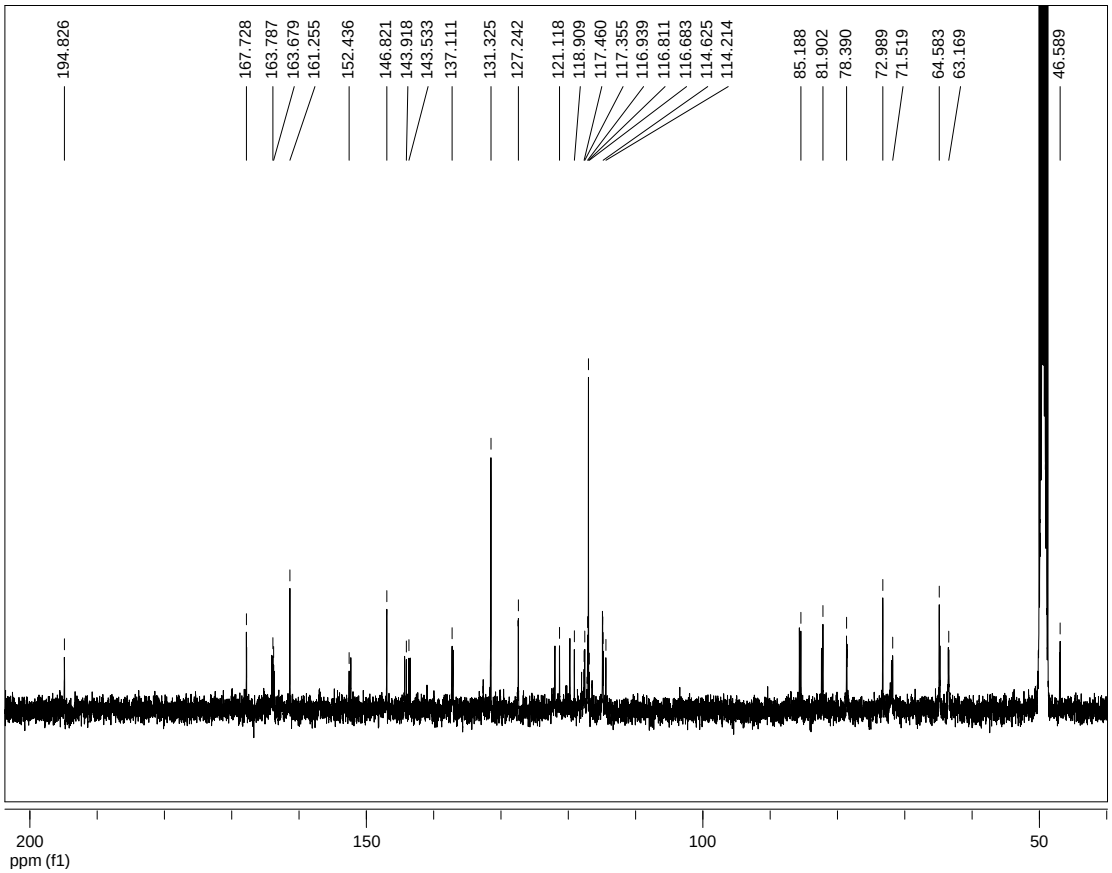
Appendix 3: IR, Mass, UV, ^{1}H NMR, ^{13}C NMR and DEPT spectra– Microdontin

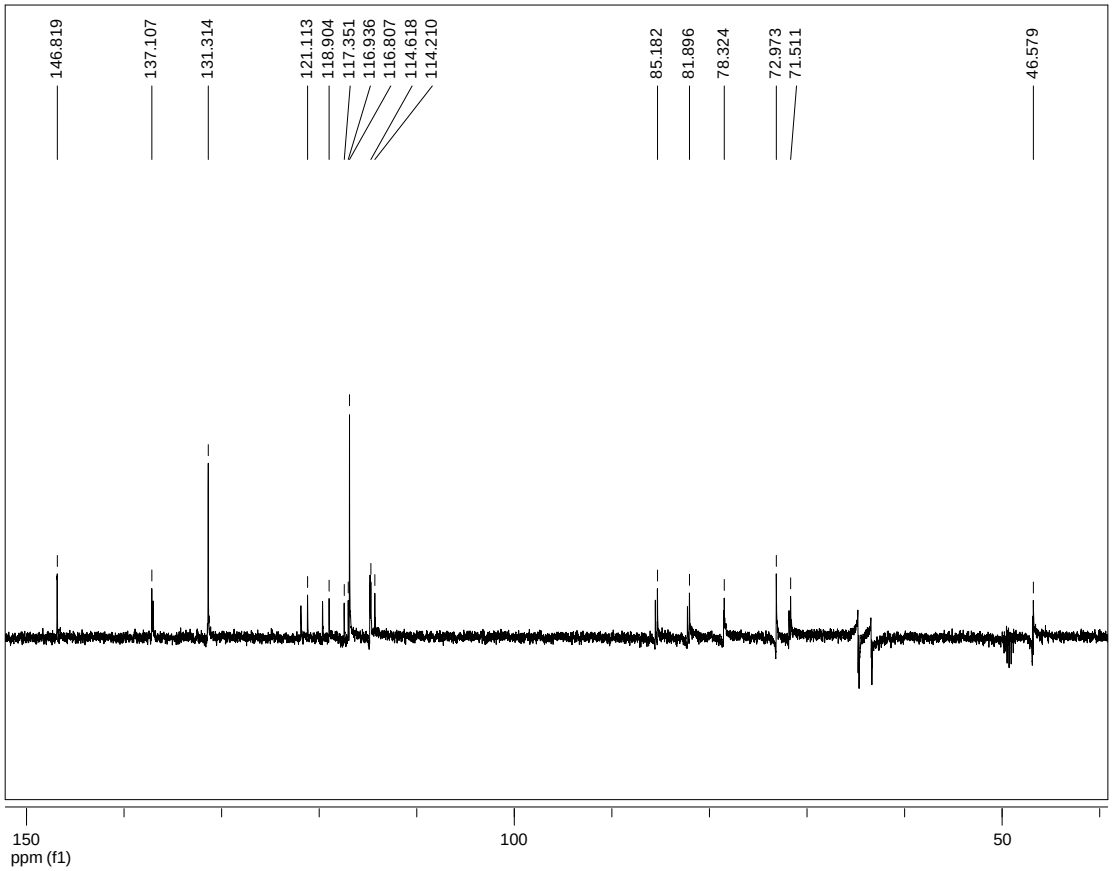




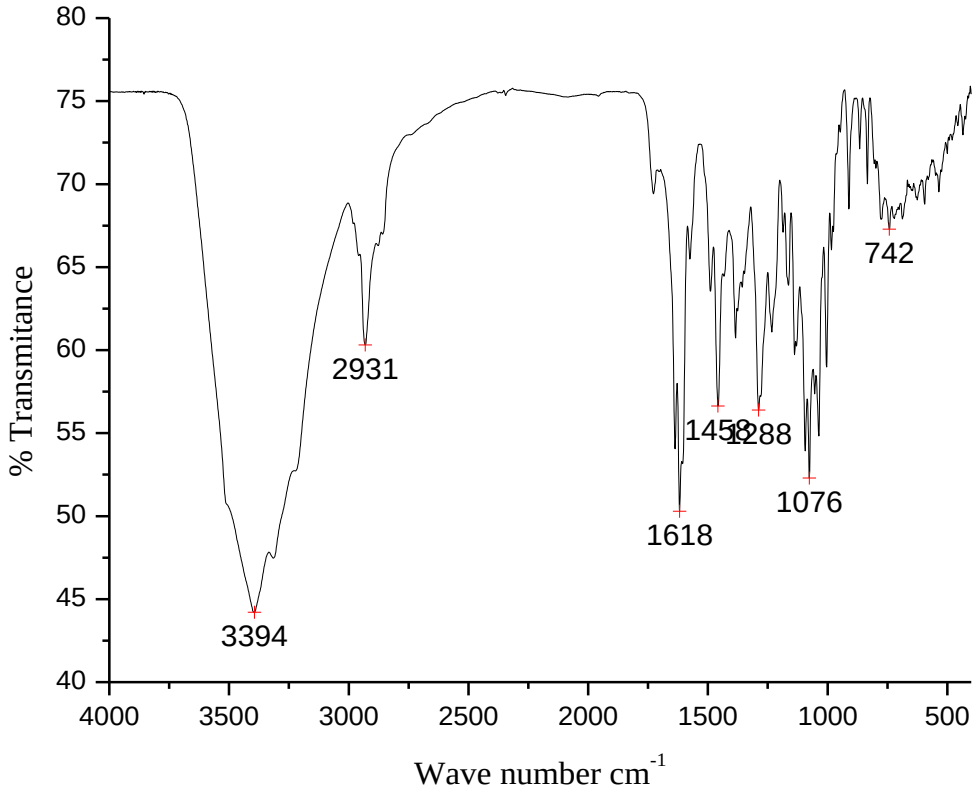


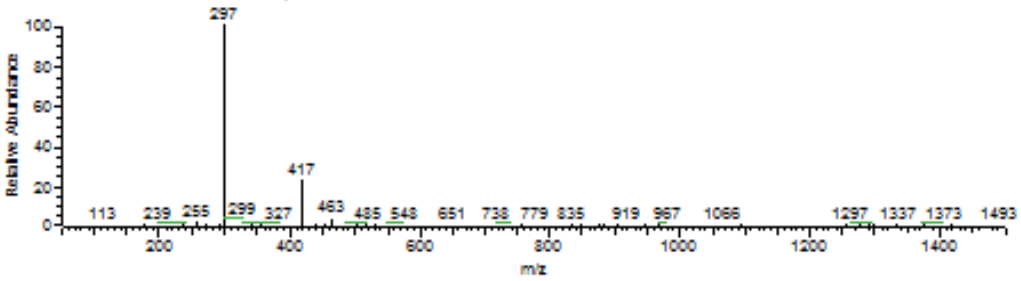

12.170
12.017
7.523
7.385
7.368
7.287
7.225
7.094
6.910
6.822
5.915
4.690
4.362
4.059
3.876
3.670
3.328
3.109
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

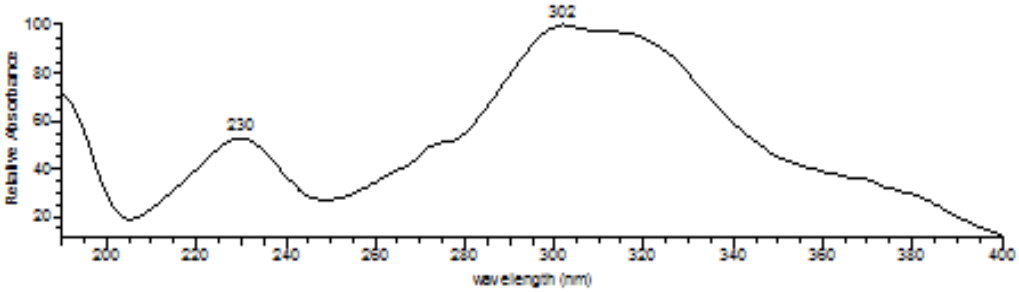

194.826
167.728
163.787
163.679
161.255
152.436
146.821
143.918
143.533
137.111
131.325
127.242
121.118
118.909
117.460
117.355
116.939
116.811
116.683
114.625
114.214
85.188
81.902
78.390
72.989
71.519
64.583
63.169
46.589
200
150
100
50
ppm (f1)

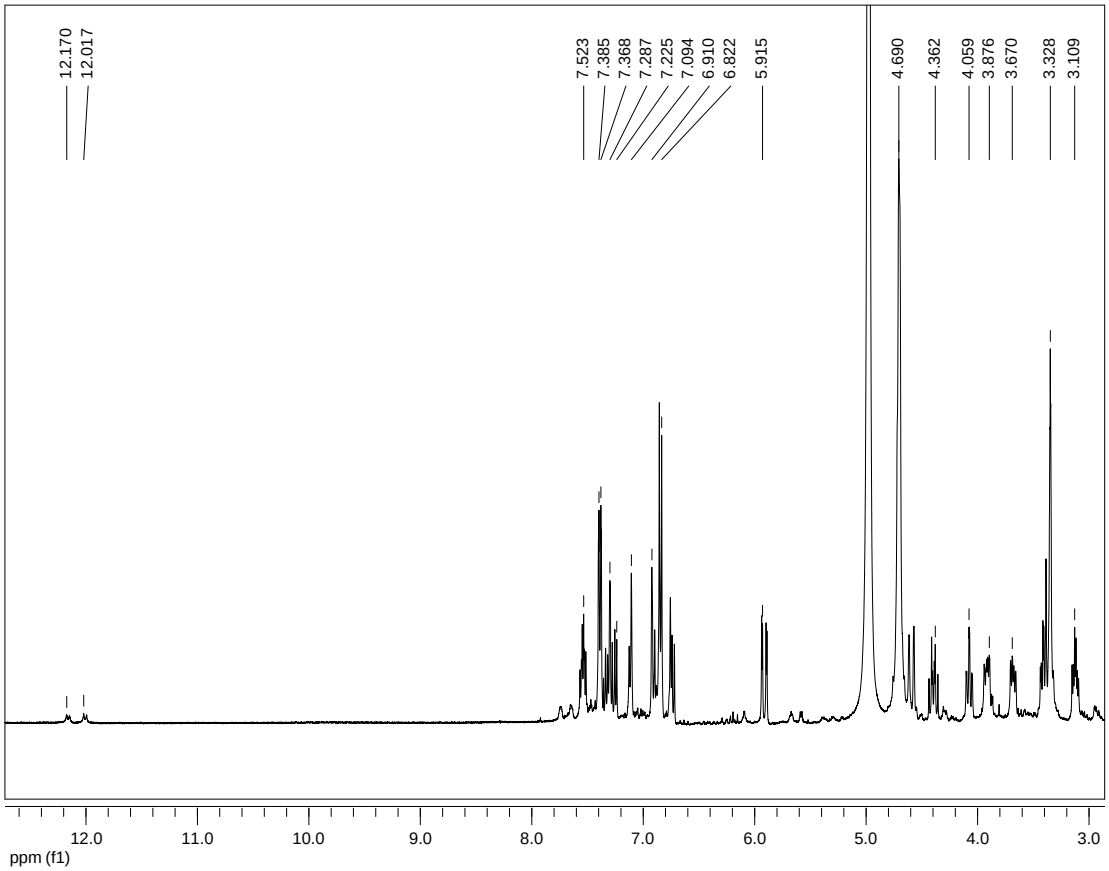

146.819
137.107
131.314
121.113
118.904
117.351
116.936
116.807
114.618
114.210
85.182
81.896
78.324
72.973
71.511
46.579
150
ppm (f1)
100
50

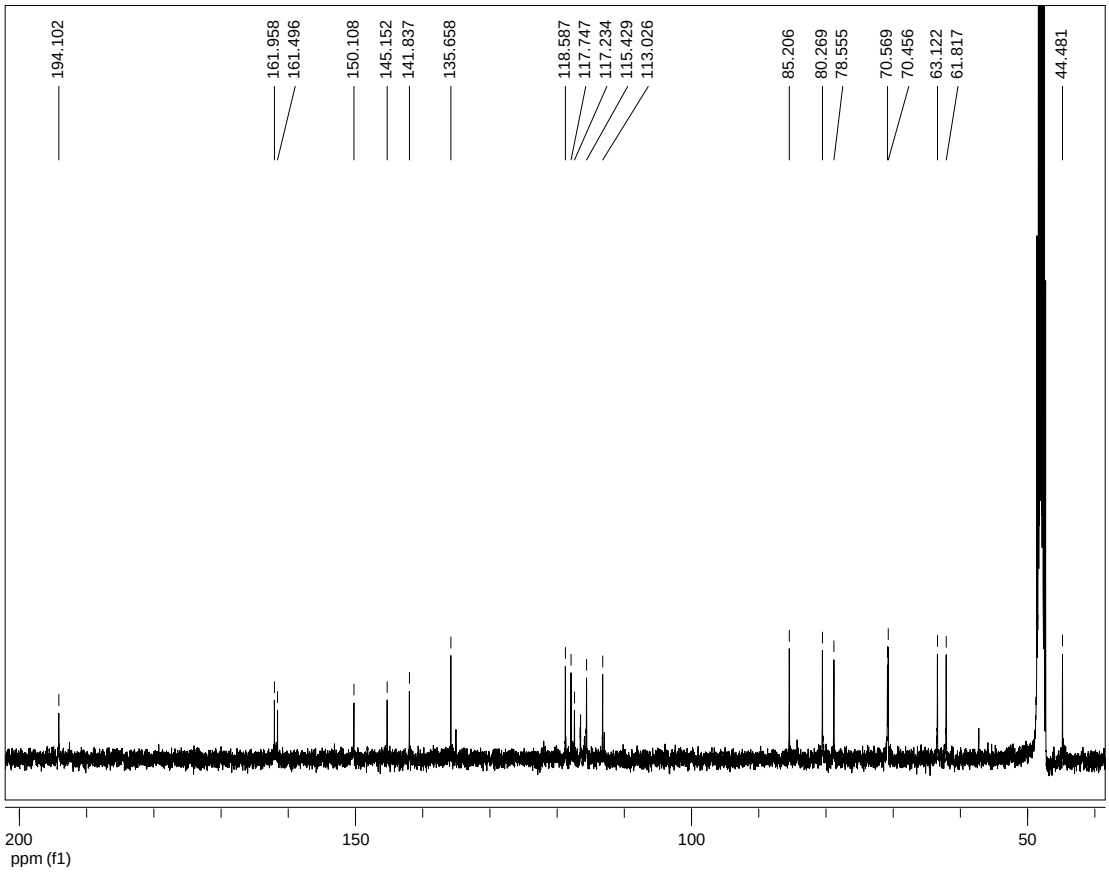
Appendix 4: IR, Mass, UV, ^{1}H NMR, ^{13}C NMR, DEPT spectra – Aloin

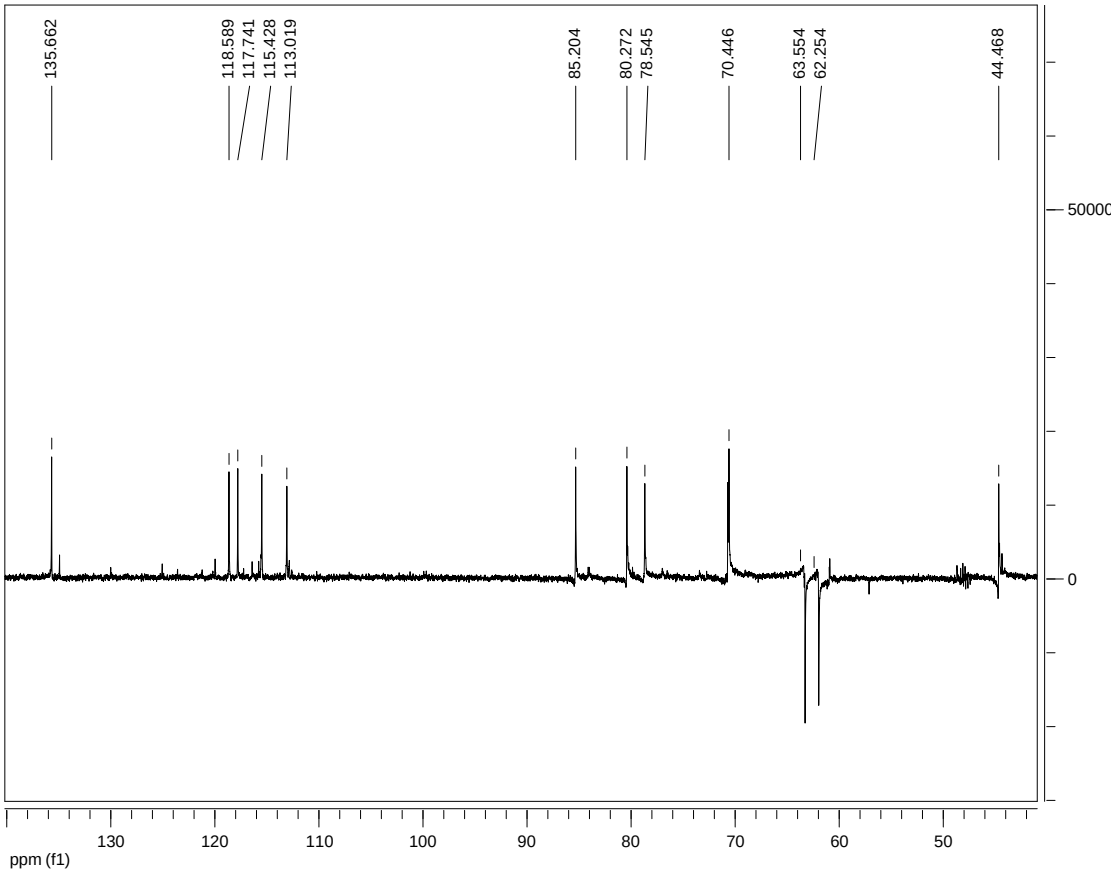




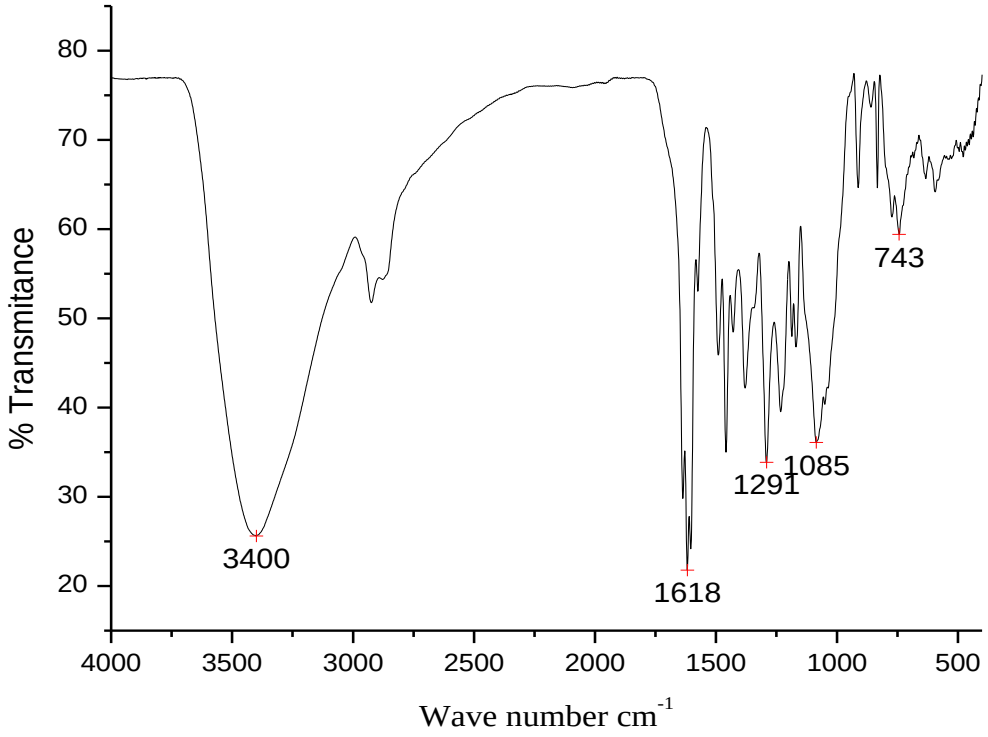


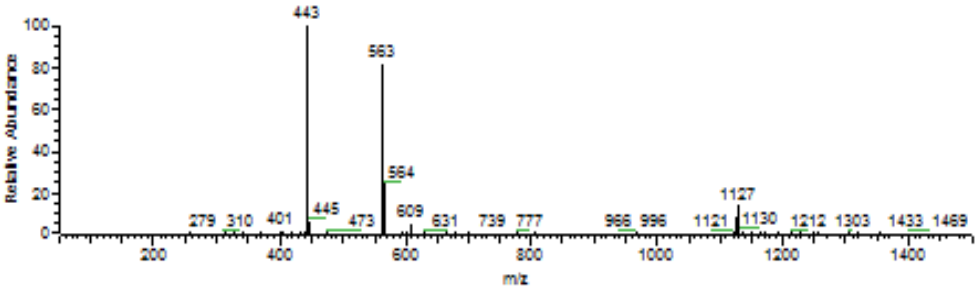

12.170
12.017
7.523
7.385
7.368
7.287
7.225
7.094
6.910
6.822
5.915
4.690
4.362
4.059
3.876
3.670
3.328
3.109
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

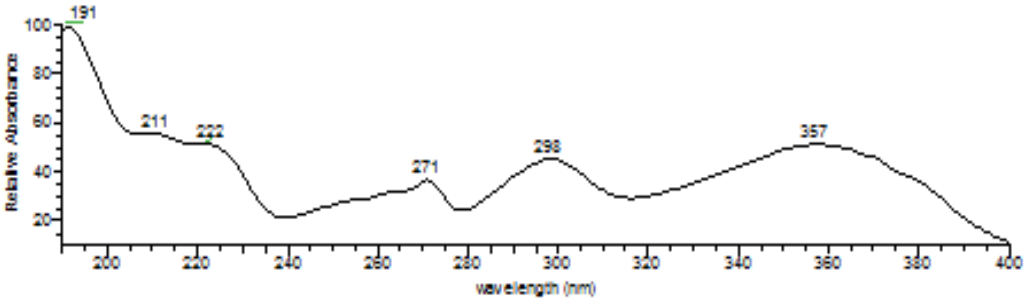

194.102
161.958
161.496
150.108
145.152
141.837
135.658
118.587
117.747
117.234
115.429
113.026
85.206
80.269
78.555
70.569
70.456
63.122
61.817
44.481
200
150
100
50
ppm (f1)

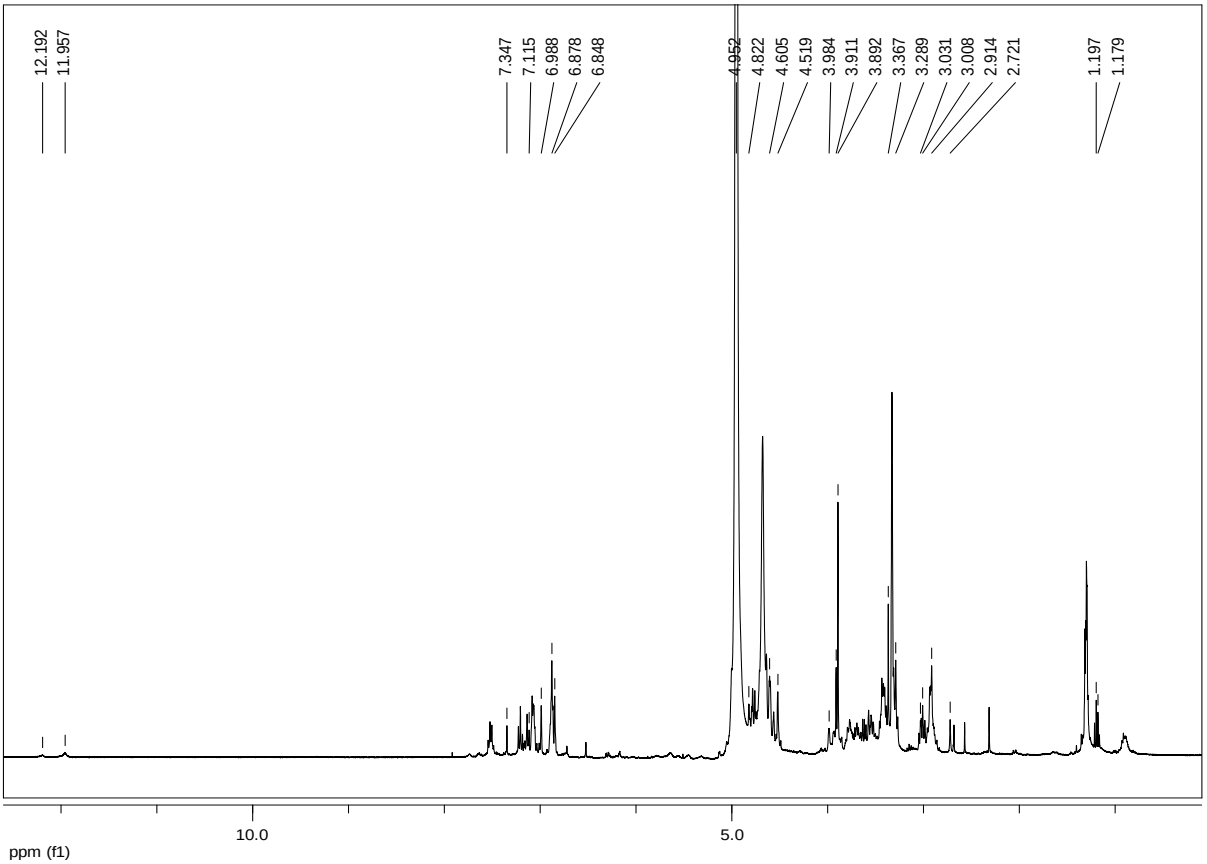

135.662
118.589
117.741
115.428
113.019
85.204
80.272
78.545
70.446
63.554
62.254
44.468
50000
0
130
120
110
100
90
80
70
60
50
ppm (f1)

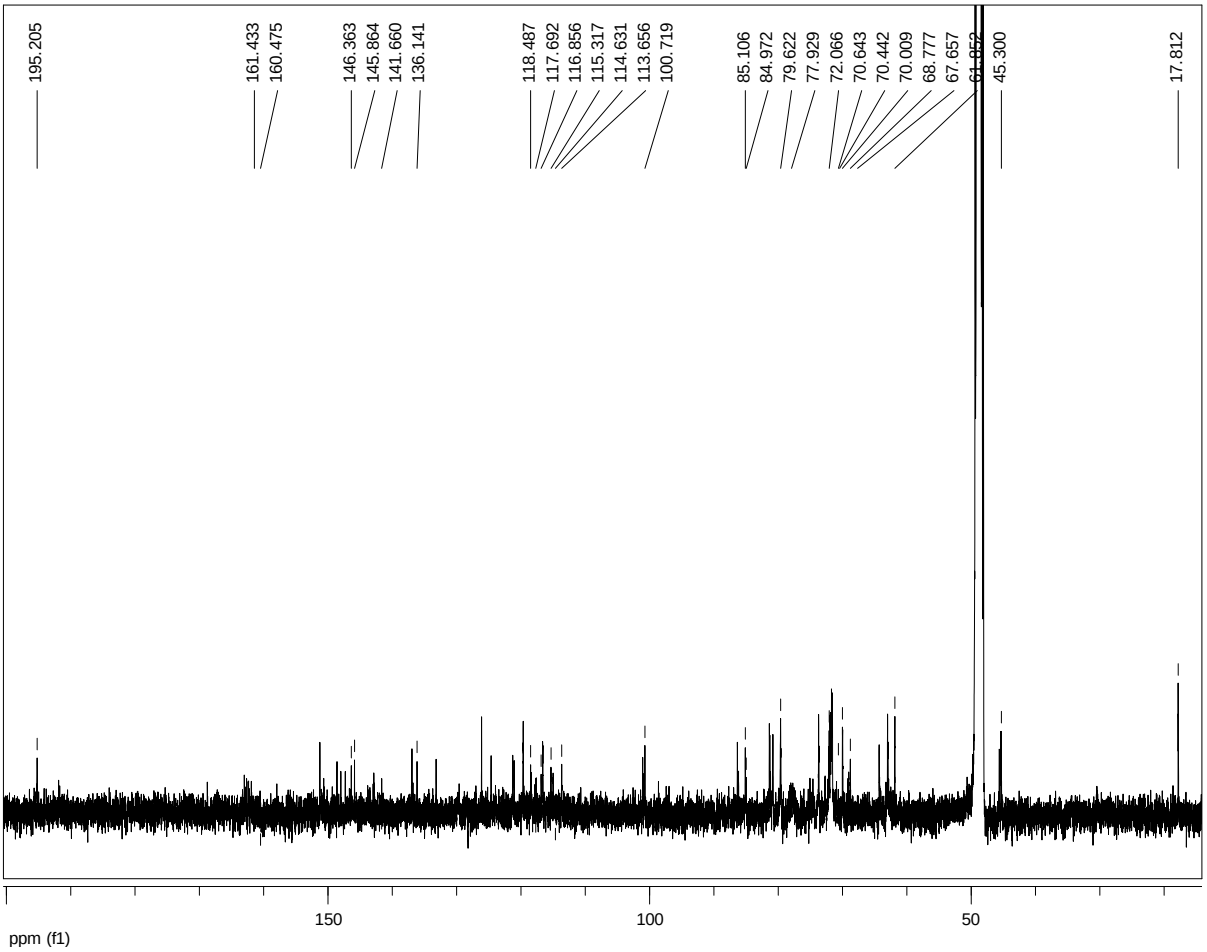
Appendix 5: IR, Mass, UV, ^{1}H NMR, ^{13}C NMR, DEPT spectra- Aloinoside

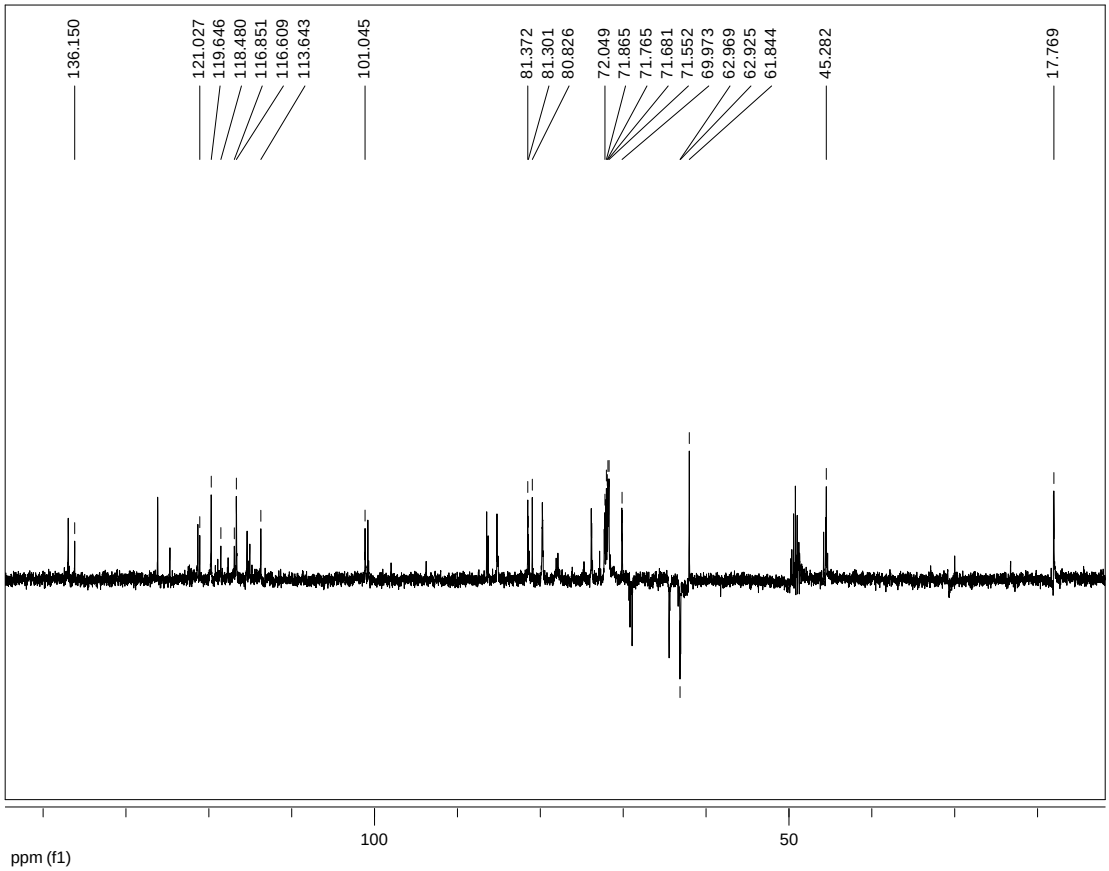







12.192
11.957
7.347
7.115
6.988
6.878
6.848
4.952
4.822
4.605
4.519
3.984
3.911
3.892
3.367
3.289
3.031
3.008
2.914
2.721
1.197
1.179
10.0
5.0
ppm (f1)


195.205
161.433
160.475
146.363
145.864
141.660
136.141
118.487
117.692
116.856
115.317
114.631
113.656
100.719
85.106
84.972
79.622
77.929
72.066
70.643
70.442
70.009
68.777
67.657
64.852
45.300
17.812
150
100
50
ppm (f1)


136.150
121.027
119.646
118.480
116.851
116.609
113.643
101.045
81.372
81.301
80.826
72.049
71.865
71.765
71.681
71.552
69.973
62.969
62.925
61.844
45.282
17.769
100
50
ppm (f1)

Appendix 6: Leaf and flowers of *Aloe sinana*

Appendix 7: Antimalarial activity of *Aloe sinana*

Appendix 8: Microscopic slide of *P. berghei*