

**ANTITRYPANOSOMAL POTENTIAL OF THE
STEM BARK METHANOLIC EXTRACT OF
HYMENOCARDIA ACIDA AGAINST
*TRYPANOSOMA EVANSI***

BY

**NWOSU, OCHEZE NNEOMA B.Sc (A. B. U 2006)
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MARCH, 2013.

DECLARATION

I declare that work in the thesis entitled: ANTITRYPANOSOMAL POTENTIALS OF THE STEM BARK METHANOLIC EXTRACTS OF *HYMENOCARDIA ACIDA* AGAINST *TRYPANOSOMA EVANSI* has been performed by me in the Department of Biochemistry Ahmadu Bello University Zaria under the supervision of Prof H. C. Nzelibe and Dr K. Anigo. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

NWOSU, Ocheze Nneoma
M.Sc/Scien/18956/2007-2008

Date

CERTIFICATION

This thesis titled TRYPANOSOMAL POTENTIALS OF STEM BARK METHANOLIC EXTRACTS OF *HYMRNOCARDIA ACIDA* AGAINST *TRYPANOSOMA EVANSI* by NWOSU OCHEZE NNEOMA meets the regulations governing the award of the degree of Master in Science (M.Sc./SCIE) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Prof. H. C. Nzelibe,
Chairman Supervisory Committee,
Department of Biochemistry

Date

Dr. K. Anigo
Member, Supervisory Committee

Date

Dr. Inuwa
Head of Department of Biochemistry

Date

Prof. Adebayo A. Joshua
Dean, Post Graduate School

Date

DEDICATION

This work is dedicated to my parents Barrister and Mrs S. Apugo-Nwosu who stood by me and encouraged me tremendously throughout the programme.

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ABSTRACT

Antitrypanosomal activities of methanolic extract of stem bark of *Hymenocardia acida* was evaluated *in vitro* and *in vivo* in *Trypanosome evansi* infected rats. *In vitro* study was carried out using three extracts (Methanol, petroleum ether, chloroform). Methanolic extract which was the most active extract was used for the *in vivo* study. LD₅₀ of the methanolic extract was calculated to be 245mg/kg. *In vivo* studies revealed that infected rats treated with methanolic extract of *Hymenocardia acida* at 90mg/kg/body weight showed significant decrease in level of parasitaemia thereby extending the survival days of the rats compared to control. In the prophylactic studies, groups given 2 and 3 doses of the methanolic extract before infection did not express parasitaemia even after 30 days post infection. Results of the phytochemical analysis showed the methanolic extract contains flavonoids, triterpines, steroids, saponins, cardiac glycosides, glycosides, tannins and alkaloids. Chromatographic studies carried out on the methanolic extract revealed 2 fractions (Fractions 1 and Fraction 2). *In vitro* and *in vivo* study of the 2 fractions revealed that fraction 2 was the most active although both fractions were able to extend the survival days of the rats compared to control. GCMS of the 2 fractions indicated presence of n-Hexadecanoic acid and Octadecanoic acid, 1-Eicosene and 17-Pentatriacontene which can be classified as simple aliphatic compounds has been implicated in the result as the active principle in the treatment of trypanosomosis.

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DEFINATION OF TERMS

Actinomorphie: Star shaped i.e. can be divided into three or more identical sectors

Alopecia: Absence of hair from where hair is present (a disease that affect hair follicles)

Anisocytosis: Red blood cells are of unequal size this is found in anaemia

Ascites: Accumulation of fluid in the peritoneal cavity causing abdominal swelling

Astringent: A substance that tend to shrink or constrict body tissues

Atrophic: Partial or complete wasting away of part of the body

Conjunctivitis: Inflammation of the conjunctiva of the eyes

Dyspnoea: Shortness of breath

Focal Polienccephomalacia: Softening of the cerebro cortical grey matter

Gastroenteritis: Inflammation of the stomach and intestines from bacteria, toxins and viral infections.

Glomerulonephitis: Acute inflammation of the kidneys typically caused by an immune response

Hemorrhoid: Are vascular structures in the anal carnal which helps with stool control, they become piles when swollen or inflamed

Human HL-60 cells: Human promyelotic leukemia cell line, is a leukemic cell line that has been used for laborathory research on how certain kinds of blood cells are formed

Hydropericardium: Accumulation of serous fluid in the pericardium

Hydrothorax: Having fluid in the Pleural cavity

Impaired motor function: Stiffness, uncoordinated movement

Jaundice: Yellowing of skin or whites of the eyes because of excess of pigment bilirubin

KB cells: Cells containing human papillomavirus 18 (HPV-18) sequences

Necrosis: Death of most or all of the cells in an organ or tissue due to disease injury or failure of blood supply

Otitis: General term for inflammation or infection of the ear in both humans and other animals

Pathognomonic: Specific characteristic or indicative of a particular disease or condition

Pokilocytosis: Abnormally shaped blood cells present in the blood

Polychromasia: A disorder where there is an abnormally high number of red blood cells found in the blood stream as a result of being prematurely released from the bone marrow during blood formation

Poultice: Soft moist mass often heated and medicated that is spread on cloth over the skin to treat an aching, inflamed or painful part of the body.

Punctuate basophila: Refers to an observation found when observing a blood smear in which erythrocytes display small dots at the peripheri. The dots represent accumulation of rRNA and it's pathological

Stiples: This are out growths bore on the either side of the base of a leaf stalk

Subserous petechial hemorrhages: An acute infectious disease characterised by pneumonia and blood infection

Urticaria: A rash of round red welts on the skin that itch intensely sometimes with dangerous swelling

MEANING OF ABBREVIATIONS

AAT: African Animal Trypanosomosis

AHT: African Human Trypanosomosis

DNA: Deoxyribo Nucleic Acid

WHO: World Health Organisation

TLC: Thin Layer Chromatography

PC: Positive Control

NC: Negative Control

KOH: Potassium Hydroxide

UV-light: Ultra Violet light

VLC: Vacuum Liquid Chromatography

GC-MS: Gas Chromatography Mass Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

Trypanosomosis is a protozoan disease of man and livestock. It is caused by trypanosomes and transmitted by tsetse fly (*Glossina palpalis*) and biting flies i.e. hematophagous flies, including *Tabanus spp.* and *Musca spp.* While *Trypanosoma brucei rhodiense* and *Trypanosoma brucei gambiense* cause African Human Trypanosomosis (AHT, sleeping sickness), *Trypanosoma brucei brucei*, *Trypanosoma vivax*, *Trypanosoma congolense*, *Trypanosoma evansi* and *Trypanosoma equiperdum* are the agents of African Animal Trypanosomosis (AAT) causing major health and commercial problems in sub-Saharan Africa.

Trypanosoma evansi, a flagellate parasite that belongs to the Trypanosomatidae family, has a worldwide distribution, affecting mainly wild and domesticated mammals. The disease which *Trypanosoma evansi* causes is known as 'surra' (Bajyana *et al.*, 1988). Research has shown that *trypanosoma evansi* is capable of infecting humans (Josh *et al.*, 2005). The acute form of the disease is usually fatal, whereas the chronic form is characterized by progressive anaemia, high fever, emaciation, edema, enlarged lymph nodes, abortion and death. Trypanosomes transmitted, severely harm human and livestock, causing serious economic losses and significantly constraining socioeconomic development in Africa.

In Northern Nigeria where trypanosomosis is prevalent, traditional healers use medicinal plants either alone or in combination to treat trypanosomosis. Thus, the search for medicinal plants with trypanocidal activities continues to generate a lot of research interests (Hoet *et al.*, 2004) especially in respect of plants with claimed efficacy but without scientific proof. However some recent reports indicate that antitrypanosomal activity exists in some medicinal plants (Abu *et al.*, 2009).

Hymenocardia acida Tul (Hymenocardiaceae) which belongs to the family Euphorbiaceae is widespread in tropical Africa with a small tree of about 6 m high. Ethnomedical investigation of *H. acida* reveals a variety of medicinal uses in tropical African countries. The bark and leaves are used together in various ways in Nigeria for abdominal and menstrual pains and as poultices on abscesses and tumors. The powdered leaves of the plant are used for the treatment of arthritis (Burkhill, 1994). *H. acida* also contains alkaloids and phenolic contents (Sofidiya *et al.*, 2009). The plant's stem bark is also reportedly used for bone setting or as an anti-inflammatory agent by traditional bone healers and in the treatment of chest pains (Muanza *et al.*, 1995).

The plant has also been screened for antitumor and anti-HIV activities (Muanza *et al.*, 1995), anti-plasmodial (Vonthron-Senecheau *et al.*, 2003), anti-trypanosomal (Hoet *et al.*, 2004) and anti-sickling (Mpiana *et al.*, 2007) with certain levels of activity recorded in each case.

1.1 STATEMENT OF RESEARCH PROBLEM

The impact of the tse-tse-associated disease extends in sub-Saharan Africa over some ten million square kilometer (a third of the continent). Of these ten million square kilometer some three million are covered by equatorial rain forest; the remaining area contains some very good grazing areas, which perhaps fortunately have been protected so far by the tsetse fly against (over)grazing. The disease threatens enormous numbers of cattle and other livestock, while some trypanosomes affect humans also directly in most of this area by causing sleeping sickness. Over this vast area the process of development as seen elsewhere has been greatly hindered (Aferwerk *et al.*, 2000).

The huge reproductive losses in livestock due to AAT are attributed to low foetal weights, premature births and neonatal losses, poor reproductive performance and poor lactation, low quality ejaculate, reduced sperm motility and concentration are other problems (Akpavie *et al.*, 1987; Faye *et al.*, 2004). Adamu *et al.* (2007) also reported trypanosome induced testicular degeneration, poor sperm characteristics and lack of libido making infected bull unfit for breeding. All these braw-backs have caused major obstacles to Agricultural development and livestock production on the African continent.

Chemotherapy is the most widely used means of controlling the disease. However, effectiveness of the drugs available is limited by a number of factors, which include increasing parasite resistance (Afewerk *et al.*, 2000) and treatment failures, unacceptable toxicity (Akanji and Ngaha, 1989; Triolo, 1990; Onyekwelu, 1999) unavailability of the drugs, logistics of administration, long period of treatment, and high cost. However, all these call for the development of new drugs (Hoet *et al.*, 2004).

1.2 JUSTIFICATION

The problem of drug resistance is ever increasing thereby creating a need to change to more efficacious drugs that are affordable and readily available. These are found in plants which are potential sources of new drugs since they contain countless number of molecules that have pharmacological effects (Newman *et al.*, 2003). Since *H. acida* is a plant used in folkloric medicine in the treatment of trypanosomosis, it becomes very necessary to investigate the effect of its extracts on *T. evansi* with a view to ascertaining its efficacy against these harmful organisms.

1.3 AIM

To investigate the antitrypanosomal potential of the stem bark extracts of *Hymenocardia acida* in *Trypanosoma evansi* infected rats

1.4 OBJECTIVES

1. To investigate *in vitro* antitrypanosomal potential of various solvents; petroleum ether chloroform and methanol, stem bark extracts of *Hymenocardia acida*.
2. To determine *in vivo* antitrypanosomal effect of the methanolic extract of stem bark of *Hymenocardia acida* in *Trypanosoma evansi* infected rats
3. To assess the prophylactic properties of the methanolic extract of stem bark of *Hymenocardia acida* in *Trypanosoma evansi* infected rats
4. To partially isolate and characterize the most active fraction of the methanolic extract using chromatographic and spectroscopic techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TRYPANOSOMES

Trypanosomes are parasites that belong to the order *kinetoplastida*, so-called because of the large DNA-containing structure, the kinetoplast, found at the base of the flagellum. The *Kinetoplastida* order of the phylum Protozoa includes two genera, *Leishmania* and the digenetic flagellates, *Trypanosoma*, members of which are responsible for most of the neglected diseases, both in man and his animals. Trypanosomes have been around for more than 300 million years (Welburn, 2001). They are microscopic unicellular protozoa that are ubiquitous parasites of insects, plants, birds, bats, fish, amphibians and mammals. Since they have been around for so long, they and their natural hosts have evolved together to ensure their mutual survival. Fortunately, few species of trypanosomes are pathogenic. Trypanosomes, and other parasites, mainly cause disease when they spread to new hosts, like humans and their domestic animals, especially recent imports into endemic areas of species that diverged since continents separated (Welburn, 2001)

Trypanosomes are generally associated with diseases in Africa and South America. Although the complex trypanosomes are causes of several veterinary diseases, only two cause significant human diseases (Barrett *et al.*, 2003). These are *Trypanosoma brucei* (*T. Brucei*), responsible for the human African trypanosomosis, also known as sleeping sickness found mainly in sub-Saharan Africa and the American trypanosomosis caused by *Trypanosoma cruzi* (*T. cruzi*) the etiological agent of Chagas' disease that afflicts millions of people in central and southern America (Westenberger *et al.*, 2005). The causative agent of the human African

trypanosomosis, *T. brucei* is further divided into two subspecies based on molecular typing. These are *Trypanosoma brucei gambiense* (*T. brucei gambiense*) which causes a chronic form of sleeping sickness in west and central Africa and *Trypanosoma brucei rhodesiense* (*T. brucei rhodesiense*) responsible for the acute form of the disease in eastern and southern Africa. There is also the natural tsetse (tsetse, being the fly vector, an insect of the *Glossina* sp. Order *Diptera*) mediated transmission of both subspecies (Barrett *et al.*, 2003). Infection with either of the subspecies is fatal if not treated. While there is also the occurrence of yet a third subspecies, *Trypanosoma brucei brucei* (*T. brucei brucei*) this species is not infectious to humans but instead infects ruminants, in addition to two other entirely different species, *Trypanosoma congolense* (*T. congolense*), and *Trypanosoma vivax* (*T. vivax*). Together, these *Trypanosoma* parasites are the causative agents of the Nagana (Stuart *et al.*, 2008) which is the trypanosomosis of the African ruminants. Another species, *Trypanosoma simae* (*T. simae*) (Luckins, 1992) is also present on the African continent; however this species causes high deaths in domestic pigs (Aksoy, *et al.*, 2003).

In common with the human parasites, the animal parasites are cyclically transmitted by haematophagous dipteran tsetse flies. Except for *T. vivax* which has a wider distribution, the exclusiveness of tsetse flies to Africa within a belt that stretches between 14° North and 29° South of the equator explains the restriction of this disease caused by *T. brucei brucei* and *T. congolense* within the sub-Saharan Africa confine where the flies are endemic. However, it is important to note that even within this zone, there are local foci which according to WHO are rapidly expanding (WHO, 1998) and this is attributable to several factors such as the presence of the animal reservoir and the tsetse vector, the ever present human to animal contact and the breakdown of infrastructure in many parts of Africa (Barrett and Okali 1999; Hide, 1999) as

well as political and economic circumstances (Cross, 2001). Another species, *Trypanosoma evansi* (*T. evansi*) with a much wider distribution found in Africa, America, Asia, Europe, the Middle and Far East is the causative agent of the animal trypanosomosis known as Surra. In contrast to the other animal trypanosomes, this species has a different mode of transmission in that it is mechanically transmitted by biting flies of the genera *Tabanus* and *Stomoxys*. In South and Central America, vampire bats may serve as vectors and reservoirs for *T. evansi*. The wider occurrence of this species according to Stephen (1986) could be attributed to the total adaptation to mechanical mode of transmission.

2.2 TRYPANOSOMA EVANSI

2.2.1 Scientific Classification

Kingdom: *Protista*

Phylum: *Euglenozoa*

Class: *Kinetoplastea*

Order: *Trypanosomatida*

Family: *Trypanosomatidae*

Genus: *Trypanosoma*

Species: *T. evansi*

Binomial name

Trypanosoma evansi

Trypanosoma evansi is a protozoan parasite of mammalian blood mechanically transmitted by biting flies of the genera *Tabanus*, *Lyperosia*, *Stomoxys* and *Atylotus* (Brun *et al.*, 1998).

2.2.2 Morphology

The flagellate is long and slender, measuring 14 to 33 μm in length and 1.5 to 2.2 μm in width. The organism is generally monomorphic, although a shorter intermediate form occasionally is observed (Brun *et al.*, 1998). This hemoflagellate has a free flagellum and a circular kinetoplast which is sometimes missing in mutated wild strains or strains that have been isolated following drug treatment. Naturally dyskineoplastic strains in which the normally high condensed DNA within the kinetoplast is reduced or absent are not uncommon and are characteristics of mechanically transmitted trypanozoon which no longer develops in tsetse flies. Additionally, *T. evansi* is covered by a thick layer of a single glycoprotein (or variable surface antigen), that is the primary immunogen eliciting antibody formation (Brun *et al.*, 1998)

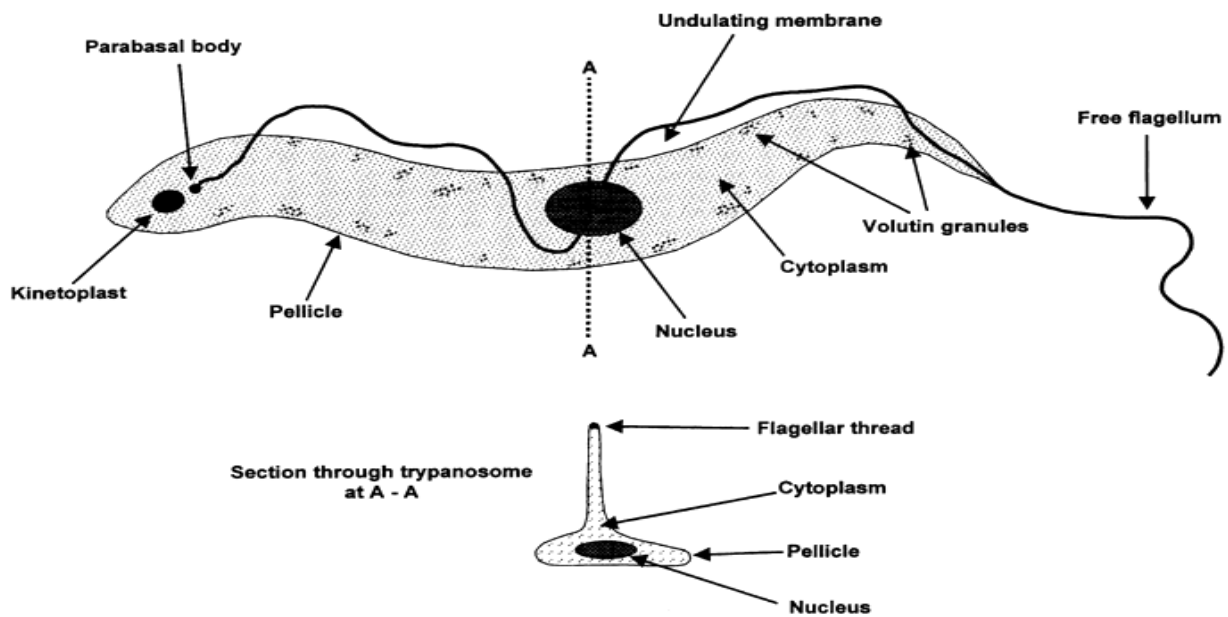


Figure 2.1 A trypanosome (*T. evansi*)

Agriculture consumer protection; A field guide for the diagnosis treatment and prevention of African animal trypanosomosis. FAO Cooperate Document Repository (www.Fao.org/./x0413E02.htm.)

2.2.3 Life Cycle

Unlike other trypanosomes, procyclic or developmental stages have not been observed in any vector. In contrast, *T. evansi* reproduces by binary fission in the mammalian host. In addition to mechanical transmission, *T. evansi* infection may be spread during nursing, copulation, or following ingestion of infected tissues by carnivores. The severity of illness depends upon the protozoal strain, concurrent infections, stress, and environmental factors (Brun *et al.*, 1998).

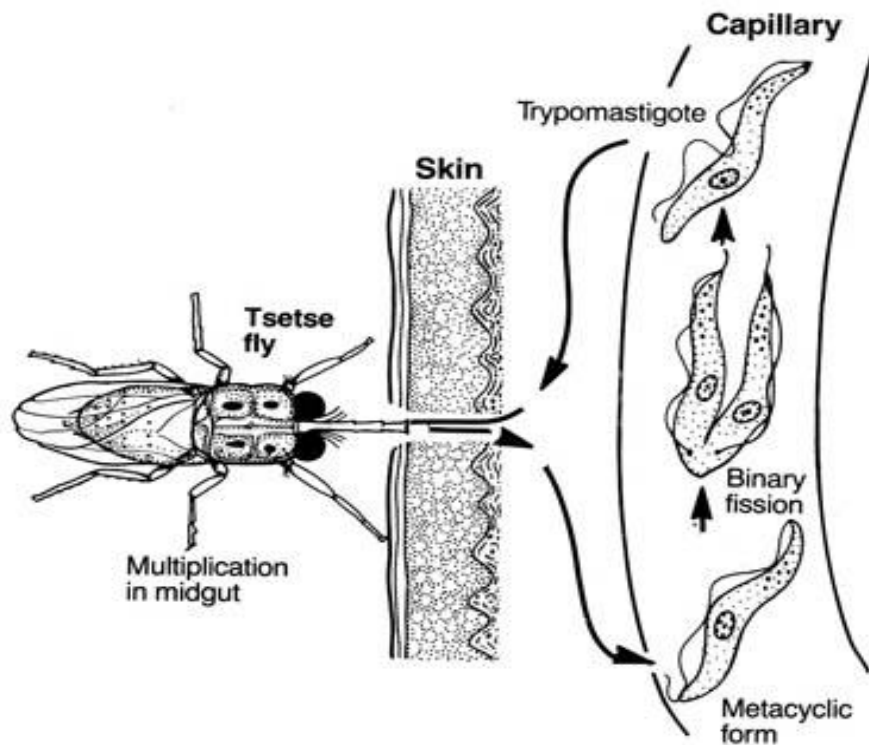


Fig 2.2 Generalized life cycle of a fly-transmitted trypanosome that reproduces by binary fission in the host (Reprinted from Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, Agriculture Handbook #651. Unites States Department of Agriculture, Washington, DC, 1988, p. 3).

2.2.4 Host Range

T. evansi affects a wide range of domestic animals (e.g. camels, equines bovine, goats, and dogs) and some wild animals, including deer, elephants, capybara and jaguars within varying degree of pathogenincity (Brun *et al.*, 1998). In china, *T. evansi* is an important pathogen of draught buffalo (Lun *et al.*,.1992a) while in South America horses and farmed capybara are severely affected. In both Sub-Saharan Africa and in North America beyond the tse –tse belt it is a major pathogen of camels and this association has been suggested as a possible route for its transportation beyond Africa and its evolution from *T. brucei* (Hoare, 1972).

2.2.5 Transmission

Tabanid flies are aggressive and vigorous attacks on the host cause defensive reactions that disturb the flies so that they attack other hosts in other to complete their blood meal. This interrupted feeding enables transmission of the trypanosomes; flies initially feeding on an infected animal may complete feeding on an uninfected host. More than 20 different species of *Tabanus* have been shown experimentally to transmit *T. evansi* (Luckins, 1999a). *Stomoxys* spp have been implicated in the dissemination of infection in Mauritius and the Philippines are also associated with transmission amongst camels in Africa. Transmission by biting flies is not the sole means by which infection is perpetuated. Ingestion of meat from infected carcass by carnivours can result in infection (Luckins, 1999b).

2.2.6 Geographical Distribution

T. evansi is the most widely distributed of the pathogenic animal trypanosomosis affecting domesticated live stock in Asia, Africa and Central and South America. Serious epidemic of

surra recorded towards the end of the 19th century and in the early years of the 20th century in Indonesia and Phillipines suggest that it could have spread into these regions in the last 100 years (Lun *et al.*, 1993; Luckins, 1999a). All species of domesticated life stock can be infected with *T. evansi* but the principal host varies geographically. In northern Africa and in parts of eastern Africa camels are the most affected host, whilst in central and South America the horse is affected. In Asia a much wider range of host is involved including Bactrian and dromedary camels, cattle, waterbuffalo horses and pigs. In Africa and South America domesticated life stock other than camels and horses are rarely affected clinically with *T. evansi* but there is serological evidence of infection in goats and sheep in Sudan and in the cattle from Brazil (Luckins, 1999a). Horses with fulminating infection with *T. evansi* are a potential source of infection for cattle or water buffalo and these in turn can act as reservoir of infection. It is not clear how important is the infection in wild animals and their relationship with domesticated animals in farm/ranch situations.

T. evansi was introduced throughout its geographical range as a parasite of domesticated livestock and since the infections in wild animals are acquired secondarily the diseases is often fatal. However, *T. evansi* shows a wide range of pathogenincity and chronic infections in wild animals do occur as in capybara in South America (Franke *et al.*, 1994).

2.3 AFRICAN ANIMAL TRYPANOSOMOSIS

2.3.1 The Disease

African Animal Trypanosomosis (AAT) as it is well known is a disease complex caused by *T. congolense*, *T. vivax*, *T. evansi*, and *T. brucei brucei* or simultaneous infection with one or

more of these trypanosomes and transmitted by tse-tse flies and biting flies. AAT is most important in cattle but can cause serious loss in pigs, dogs, camels, goat, horses, donkeys, mules and sheep. Infection of cattle by one or more of the African animal trypanosomes result in sub acute, acute or chronic disease characterized by intermittent fever, anemia, occasional diarrhea and rapid loss of condition which often terminates in death (Finelle, 1973).

‘Surra’, is of Indian origin which means “heavy breathing from the nostrils”. The disease is also known in many Arabic speaking part of Africa as ‘el dedab’ and many other local names exist e. g. ‘gufer’. In Sudan the disease occurs where ever camels are reared. It is also of economic importance in Asian countries (domestic) with buffalo and to a less extent in some countries in cattle as well (Uilenberg, 1998).

2.3.2 Pathogenesis

Initial replication of trypanosomes is at the site of inoculation in the skin: this causes a swelling and a sore (chancre) when parasites are inoculated intradermally into the mammalian host. Since the number of trypanosomes carried on the proboscis of biting flies is low, it is possible that an early phase of multiplication in the skin serves to increase both the population and the antigenic diversity of the parasites that enter the bloodstream. The multiplying trypanosomes and the resultant intense cellular infiltration in the skin may promote the induction of immunity. Trypanosomes then spread to the lymph nodes and blood then continue to replicate. *T. evansi* localize in the tissue. Antibody developed to the glycoprotein coat of the trypanosome kills the trypanosome and result in the development of immune complexes. Antibody however does not clear the infection, for the trypanosomes have genes that can code for many different surface coat glycoproteins and change its surface glycoprotein to evade the

antibody (Donelson, 2003). Thus there is a persistent infection that results in a continuing cycle of trypanosomes replication antibody production, immune complex development and changing surface glycoprotein.

Immunological lesions are significant in trypanosomosis and it has been suggested that many of the lesions (e. g anemia and glomerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with or prevent normal organ function (Edwards, 1956). The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression that occurs following infection by these parasites. These marked immunosuppression lowers the host's resistance to other infections and thus results in secondary disease which greatly complicates both the clinical and pathological features of trypanosomosis (Ikede *et al.*, 1972).

2.3.3 Clinical Signs

Typically trypanosomosis is a wasting disease in which there is a slow progressive loss of condition accompanied by increasing anemia and weakness to the point of extreme emaciation collapse and death. In Indonesia, *T. evansi* infections in cattle and water buffalo are typically chronic with associated weight loss and anaemia. A wide range of other clinical signs have been attributed to *T. evansi* infection in cattle and water buffalo, including fever, salivation, diarrhoea, oedema, jaundice, conjunctivities, lacrimation, mucopurulent nasal discharge, dyspnoea, alopecia, urticaria, swelling of superficial lymph nodes, weakness, incoordination and paralysis (Luckins, 1999b). Antigen-antibody complexes formed during the host response to *T. evansi* infection may cause inflammatory reactions in the central nervous system, myocardium and skeletal muscles. Also invariably present are intermittent fever and

general weakness. Abortion may be seen and infertility of males and females may be sequel. The severity of clinical response is dependent on the species and the breed of affected animal, and the dose and virulence of the infecting trypanosome. Stress such as poor nutrition or concurrent disease plays a prominent role in the disease process and under experimental condition where stress may be markedly reduced, it is difficult to elicit clinical symptoms. There are also differences between infections caused by various species and host populations or breeds and it must be stressed that there are great differences under the influence of various circumstances as listed above (Unilenberg, 1998).

In camels, *T evansi* infection is sometimes complicated by pulmonary infections, striking clinical symptoms develop a month or so after infection with acute bouts of fever associated with dullness lack of appetite and lachrymation, coinciding with peaks of high parasitemia. Gradually the animal losses condition, the hump shrinks and progressive weakness becomes noticeable. Oedema may occur; pregnant animals often abort. The disease is usually fatal, sometimes rather quickly, a few weeks or more often a few months or more after the onset of the disease the evolution is much more chronic and may last as long as 2 or 3 years. In horses the African and Asian forms of the disease are severe; Oedema and impaired motor function are usual (Unilenberg, 1998).

2.3.4 Gross Lesions

No pathognomonic change is seen in AAT. Anemia, oedema, and serious atrophy of fat are commonly observed. Subcutaneous oedema is particularly prominent and is usually accompanied by ascites, hydropericardium and hydrothorax. The liver may be enlarged and edema of lymph nodes is often seen in the acute disease, but they may be reduced in size in the

chronic disease. The spleen and lymph nodes may be swollen normal or atrophic. Necrosis of kidneys and heart muscle and subserous petechial hemorrhages commonly occur. Gastroenteritis is common, and focal poliiencephalomalacia may be seen. A localized lesion (chancre) may be noted at the site of fly bite, especially in goats. The anemic blood changes are anisocytosis, poikilocytosis, polychromasia, and punctuate basophilia (Moulton, 1976).

2.3.5 Diagnosis

Trypanosomosis should be suspected when an animal in an endemic area is anemic and in poor condition. Confirmation depends on the demonstration of the organism in blood and lymph node smears (Kuzoe, 1991).

In the early phases of infection, the parasite can readily be observed by microscopic examination of a wet mount of blood slides. Wet unstained blood films are examined by light microscopy using 400 × powers to detect the motile trypanosomes. Thin smears fixed in methyl alcohol can be stained with giemsa or mayrunwald stain and they are favoured for species identification. Thick smears are air dried and stained with Giemsa and also examined by light microscopy and examined at high magnification. Thick films have the advantage of being able to detect parasites in low numbers however; the morphology of the parasite is difficult to determine (Unilenberg, 1998).

Trypanosomes can be difficult to find in the blood especially in animals that are healthy carriers (Kuzoe, 1991). Detection can be improved by parasite concentration techniques including mini anion-exchange chromatography hematocrit-centrifugation, the quantitative buffy coat method or the dark-ground/phase-contrast buffy coat technique. The latter three

methods rely on the concentration of trypanosomes near the buffy coat after centrifugation (Unilenberg, 1998).

2.3.6 Control and Eradication

2.3.6.1 Vector Control

Fly eradication and drug Prophylaxis are the only effective trypanosomosis control methods presently available. Several approaches to fly control have been used with varying degree of success. Also preventive measures used by lives stock owners to discourage attacks by biting flies includes stabling animals, use of smudge fires and installation of netting to protect dairy cattle. It is not known how effective these measures might be in reducing transmission of trypanosomosis (Luckins, 1999b).

2.3.6.2 Chemotherapy and Chemoprophylaxis

Strategies for control have heavily relied on the use of chemotherapy to treat infected animals. It is generally recognized that chemotherapy is most effective in the early stage of infection. Four compounds, namely suramine, dimenazine aceturate, isometamedium and quinapyramine, have been used to treat camels, cattle, water buffalo, horses and pigs infected with *T. evansi* for many years (Luckins, 1999b).

Indeed Suramine has been the main stay for over 70years even though its intravenous route of administration can be problematic in the field. It is a colourless, polyanionic sulfonated naphthylamine which is highly soluble in water. Suramine is universally effective at a dose of 10mg/kg, slowly trypanocidal, eliminating parasites from blood and lymph nodes 12-36hrs after injection. As a large polyanion it inhibits numerous enzymes, such as L-glycerophosphate

oxidase, glycerol-3-phosphate dehydrogenase, RNA polymerase kinases, thymidine kinase, dihydrofolate reductase, hyaluronidase, urease, hexokinase, fumarase, trypsin, reverse transcriptase and receptor-mediated uptake of low density lipoprotein (LDL) by trypanosomes (Wang, 1995), although whether any of the reported effects influences trypanocidal activity is unknown. Suramine is taken up by trypanosomes by pinocytosis, as a plasma protein bound complex (Fairlamb and Bowman, 1980). The accumulation of Suramine in the trypanosomes was hypothesized to be one of the reasons for the different toxicity between host and parasite. Immediate life-threatening side effects include collapse, with nausea, vomiting and shock. Severe delayed reactions include kidney damage, haemolytic anaemia, jaundice and severe diarrhoea, all of which can be fatal (Fairlamb, 2003; Ian *et al.*, 2004 and Bacchi, 2009). However action of Suramine is slow. Suramine can confer only limited protection of about one month to the treated animal.

Dimenazine aceturate has been used at dose rates of 3.5mg/kg/body weight by intramuscular injection to cure *T. evansi* in cattle, water buffalo and donkeys in India. It is an aromatic diamidine and readily soluble in water however, its apparent low incidence of adverse reactions and significant therapeutic activity has led some physicians in endemic countries to use it extensively (Bacchi, 2009). It is very cheap, regularly produced and easily obtainable. The mode of action is not certain but it is partially through irreversible inhibition of trypanosomal S-adenosyl-methionine-decarboxylase, an enzyme involved in the synthesis of trypanosomes (Ian *et al.*, 2004).

The trypanosome kinetoplast is the primary site of isometamidium accumulation. The main mode of action of the drug is the cleavage of kDNA-topoisomerase complexes. Isometamidium

is seen as a 'hybrid molecule' which exhibits some of the properties of homidium and diminazene. Moreover, the drug product of isometamidium as marketed contains 70% isometamidium, and the remaining fraction (30%) is a mixture of its two isomers, a small proportion of a bis-compound and homidium (RMB Animal Health). Some studies on *T. evansi* and *T. equiperdum* have shown that the minimum effective dose of isometamidium which killed trypanosome population by 100% within 24 h of drug exposure was 1-4 µg/ml (Zhang et al., 1991), and with 96 h drug exposure, 1-300 ng/ml (Brun and Lun, 1994).

Quinapyramine is used at 2-5 mg/kg by sub-cutaneous injection in cattle, camels and horses. Its mode of action is it causes a kinetoplastic DNA condensation. It is the preferred drug used in India. It is now produced mainly for the treatment of surra in camels and horses, in particular where there is resistance of *T. evansi* to suramin. It is dispensed as a pale cream powder, producing a clear solution in water. Toxicity in horses and dogs is also well known (Biobaku et al., 2008).

Drug resistance is known to occur amongst *T. evansi* isolates and there have been reports of its occurrence in several different countries in Africa and Asia. Drug resistance has been reported from Kenya where epidemiological studies using in vitro techniques revealed stocks of *T. evansi* resistant to Suramine and quinapyramine. Resistance to one or more of the trypanocidal drug used in cattle has been reported in at least 13 countries of sub-Saharan Africa (Greets and Holmes, 1998). Quinapyramine usage has also shown to induce resistance to isometamidium, homidium and diminazene.

Chemotherapy, the main means of controlling the disease is undergoing a lot of problems due to parasite resistance (Maser *et al.*, 2003), limited and expensive trypanocidal drugs and also toxicity due to long period of treatment (Amaechi, 2001). The poor prospect for a vaccine due to antigenic variation of the parasite (Nantulya & Molloo, 1989) is further compounded by unwillingness of the pharmaceutical industry to develop new compounds because of uncertain and unprofitable market or perhaps the localized nature of the disease. Herbal preparations for the treatment of several diseases still hold a strong position in rural areas. In Northern Nigeria where trypanosomosis is prevalent, traditional healers use medicinal plants either alone or in combination to treat both human and animal trypanosomosis.

2.4 MEDICINAL PLANTS

Medicinal plants are plant or plant parts used for its medicinal, aromatic or savory qualities and are used as a major source of drugs for the treatment of various health disorders. There are a total of some 250,000 species of higher plants in the world (Cavin, 1998). The plant kingdom has contributed immensely to the health need of man when no synthetic drugs were available. Even today almost 25% of all prescribed medicines in the developing world contain ingredients derived from medicinal plants (Cordell, 1991). The world has witnessed growing scientific and commercial interest in medicinal plants, mainly due to their immense economic potential and wide spread cultural acceptability of plant based products. An inventory of medicinal plants compiled by WHO on the bases of literature from 91 countries including the classical text on Ayurvedic and unami medicine list 21,000 species of medicinal plants (Farnsworth, 1985). According to the pharmacologist Norman Farnsworth of the University of Illinois in USA, there are 89 plant- derived drugs prescribed in the industrialized world According to a WHO

estimate around 80% of the world's 5.76 billion populations in the developing world relies on herbal remedies for their basic health care needs (Fransworth, 1985). In the last 100 years, many important discoveries have been made. Medicinal plants were examined carefully and many substances were extracted from different parts of plants (Cordell, 1991).

A medicinal plant may contain one or many different compounds having medicinal activity. This pure compound may be used or mixed together to make very effective medicines (Steiner, 1986). Medicinal plants have been used in the Islamic period and other civilizations for the cure of various diseases. Medicinal plants have long been subjects of curiosity and need. Plants have been used as medicine from the initiation of human existence. Herbal medicines are always in the form of vegetable drugs or their extracts which primarily serve for the treatment of diseases and to maintain health or are utilized primarily by man for the treatment of disease or to maintain a state of improved health. Even in this era of antibiotics, radiotherapy, and several drug laws, very large quantities of herbal products are consumed and marketed throughout the world as health nutrients, exotic food supplements, preventive drugs, or actual medicines (Wall, 1969).

The study of plant medicines was first prescribed precisely in the basic literature of Charak Samhita of Ayurvedic medicine in India. Charak, the author of Charak Samhita, flourished in the 8th century BC (Bhakuni *et al.*, 1969). By the 16th century, a fundamental turning point was reached in the history of medicinal plants with the advent of the Swiss-German physician Paracelsus (1533-1541), who gave the concept of what is now defined as the "active principle" of plants (Steiner, 1986).

Western medicine has largely confined itself to the isolation of synthesis of single active ingredient for the treatment of specific disease (Bland, 1983). With the development of medicinal chemistry in the early 19th century, plants were also found as sources of substances to be developed as drugs. Presently, in spite of the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation, 25% of prescribed medicines in industrialized countries are of plant origin and some 120 plant derived compounds are used in modern therapy (Foye, 1995).

Many of our present medicines e.g. quinine, are derived directly or indirectly from higher plants. In 1785, Whitering published the use of *Digitalis purpurea*, and this eventually led to the isolation of digoxin, a cardiotonic agent (Cragg *et al.*, 2002). In 1816, Sertürner isolated the analgesic, morphine, from *Papaver somniferum*, and the isolation of the antimalarial drug, quinine, from the bark of *Cinchona pubescens* was reported in 1820. Some other discovery highlights were the isolation of atropine from *Atropa belladonna* in 1831, cocaine from *Erythroxylum coca* in 1860, and ephedrine from *Ephedra sinica* in 1887 (Buss *et al.*, 1995). The alkaloids vinblastine and vincristine from the Madagascar periwinkle (*Catharanthus roseus*) became available in the 1960's and are now extensively used in the treatment of different types of cancer (Neuss *et al.*, 1990). Another promising anticancer drug is taxol, which was isolated from *Taxus brevifolia* in 1971. In the early 1970's artemisinin, a potent and essentially non-toxic antimalarial agent was isolated from *Artemisia annua*, a Chinese medicinal plant (Klayman, 1993). In addition to purified plant-derived drugs, there is an enormous market for crude herbal medicines as dietary supplements, and for therapeutic purposes in both the developed and developing countries of the world (De Smet, 1997).

Interest in medicinal plants as a re-emerging health-aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs (Newman, 2003). A combination of this fact with the school of thought that believes that emergence/re-emergence of serious and costly infectious diseases might best be met with new anti-infective agents from traditional plant remedies (Camacho *et al.*, 2000) has greatly increased the awareness and use of herbals/traditional plant remedies. While medicinal plants and traditional medicine are integral parts of the health delivery system in developing societies like Nigeria where there is a heavy reliance on such, the developed countries too have in recent times turned to the use of traditional medicinal systems that involve the use of herbal drugs and remedies.

Although, In spite of these rapid developments in scientific technology and better understanding in the chemistry of natural products, it is fair to say that of the 6000 or more plants used in different traditional system of medicine, only a few hundred have so far been examined in depth for their chemical constituents and physiological activity. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable resource to be investigated (Anon, 1975, 1985; Hamburger *et al.*, 1991; Potterate *et al.*, 1995). The evaluation of crude drug which eventually noticed the commercial market is of considerable importance. Physical and biomedical characteristic of drug formed the main features of the study in earlier age than since the latter half of the 19th century, the emphasis was laid on crude drug, their substitutes, fluorescence analysis, preliminary phytochemical tests and adulterants, for the reason that of the commercial practices such compiled descriptions are given in the British pharmacopoeia, British pharmacopoeia commission and other pharmacopoeias.

2.5 ANTITRYPANOSOMAL PROPERTIES OF MEDICINAL PLANTS

In the search for new trypanocides, a wide range of medicinal plants have been screened for antitrypanosomal activity and quite a number of them have been reported to have significant antitrypanosomal activity (Ogbadoyi *et al.*, 2007; Nathan *et al.*, 2011). This is due to the fact that these plants contain phytochemicals which are responsible for this activity. Such phytochemicals include alkaloids, flavonoids, terpenoids and quinones. A lot of plants belonging to different families have been known to have antitrypanosomal activity both *in vitro* and *in vivo* (Atawodi *et al.*, 2003; Atawodi *et al.*, 2004; Wurochekke *et al.*, 2004; Ene *et al.*, 2009; Abu *et al.*, 2009; Adeiza *et al.*, 2009; Olukunle *et al.*, 2010; Umar *et al.*, 2010) due to the fact that they've been used for the treatment of trypanosomosis traditionally.

Ene *et al* (2009) reported on the antitrypanosomal effect of petroleum ether, chloroform and methanol extracts of *Artemisia maciverae* Linn. The chloroform extract which showed the highest activity both *in vitro* and *in vivo* assessments were subjected to bioassay guided fractionation which showed presence of phtochemicals and high antitrypanosomal activity both *invitro* and *invivo*. This report shows that the Phytochemical are indeed responsible for the antitrypanosomal effect of the extracts. (Ene *et al.*, 2009).

Atawodi *et al* (2003) reported on the antitrypanosomal properties of Methanol extracts from twenty three plants harvested from the Savannah vegetation belt of Nigeria which were analyzed *in vitro* for trypanocidal activity against *Trypanosoma brucei brucei* and *Trypanosoma congolense*. Some extracts were strongly trypanocidal to both organisms while others were trypanocidal to either *T. brucei brucei* or *T. congolense*. Also the *in vitro* trypanocidal activity of 13 medicinal plants used by local herdsmen in Northern Nigeria for the treatment of trypanosomosis was investigated. Forty-four different extracts prepared from the

13 plants were screened for *in vitro* activity against *Trypanosoma brucei brucei*. Four of the extracts showed activity against the parasite at minimum concentration of 8.3 mg/ml of blood (Wurochekke *et al.*, 2004).

Abu *et al* (2009) reported on the *in vitro* antitrypanosomal activity of crude extracts of some Nigerian medicinal plants; results showed that *Hymenocardia acida* extracts were active against *Trypanosoma brucei brucei* at minimum inhibitory concentration of 2.5 mg/ml. While the antitrypanosomal activity of *Gardenia erubescens* and *Lophira lanceolata* were effective at minimum inhibitory concentration of 20 mg/ml.

Essential oils (EOs) from *Cymbopogon citratus* (CC), *Eucalyptus citriodora* (EC), *Eucalyptus camaldulensis* (ED), and *Citrus sinensis* have been evaluated *in vitro* for activity against *Trypanosoma brucei brucei* (Tbb) and *Trypanosoma evansi* (*T. evansi*). The EOs were found to possess antitrypanosomal activity *in vitro* in a dose-dependent pattern in a short period of time. The drop in number of parasite over time was achieved at doses of 0.4 g/ml, 0.2 g/ml, and 0.1g/ml for all the EOs. The concentration of 0.4 g/ml *Cymbopogon citratus* was more potent at 3 minutes and 2 minutes for *T. brucei brucei* and *T. evansi*, respectively (Nathan *et al.*, 2010). Also during the hydrodistillation of the fresh leaves of *Hyptis spicigera* a colourless volatile oil with yield of 0.65% was gotten and the volatile oil gave forty compounds on the basis of Gas Chromatography-Mass Spectroscopy (GC-MS) with low composition of cineole (4.11%) and caryophyllene (2.61%) while α -pinene (12.16%) β - pinene (9.47%) and α -phellandrene (10.19%) were predominant compounds. The biological activity of the volatile oils was evaluated *in vitro* for activity against *Trypanosoma brucei brucei* (Tbb) and was found

to possess anti-trypanosoma activity *in vitro* in a dose dependent pattern. This activity showed the volatile oils from *H. spicigera* leaves to be a potential trypanocide.

The *in vitro* and *in vivo* antitrypanosomal activity of *Xantium strumarium* leaves against *Trypanosoma evansi* has also been studied and the ethanolic extract exhibited trypanocidal activity. The minimum inhibitory concentration (MIC) value was found to be 5µg/ml and for the *in vivo* the survival of the experimental animal was significant. The methanolic extracts of the leaves, stem and root of *Prosopis Africana* has also been reported to show strong *in vitro* and *in vivo* antitrypanosomal activity against *trypanosoma brucei brucei*. The minimum inhibitory concentration for the *in vitro* studies was found to be 4mg/ml and the dose to which it had an *in vivo* antitrypanosomal effect was 200mg/kg (Atawodi *et al.*, 2004).

The *in vitro* antitrypanosomal activity of methylene chloride, methanol and aqueous extracts of the leaves and twigs of five plant species (*Cassia sieberiana* , *Hymenocardia acida* , *Pericopsis laxiflora*, *Strychnos spinosa* , *Trichilia emetic*) traditionally used in Benin for the treatment of sleeping sickness were evaluated on *Trypanosoma brucei brucei* Determination of the IC₅₀ values of the methylene chloride leaf extracts on two strains of trypanosomes (*T. b. brucei* and *T. b. rhodesiense*) showed that all extracts possessed some antitrypanosomal activity with IC₅₀'s ranging from 1.5 to 39_g/ml (Hoet *et al.*,2004).

Morinda morindiodes, *Tithonia diversifolia*, *Acalypha wilkesiana* have been reported to have antitrypanosomal effect *in vivo* (Olukunle *et al.*, 2010) and there was significant reduction in

parasitaemia ($P < 0.05$) on the 3rd day of treatment in rats treated with *Morinda morindiodes*, *Tithonia diversifolia* and *Acalypha wilkesiana* but parasitemia later increased till survival time. Some plants known to have anti malarial effect have been reported to have antitrypanosomal effect. *Morinda morindiodes*, a plant well known for its potents antimalarial effect, had its root bark extracts exhibiting the highest value of mean survival time (12.6+0.7) days in this study. The result may probably suggest reduction in parasite virulence by *Morinda morindiodes* root bark extract.

The activity of some medicinal plants used in Sudan was tested against *Trypanosoma evansi* in vivo, they include; *Tinospora bakis*, *Argemone maxicana* and *Aristolochia bracteolate*. The three plants were selected on the basis of information from traditional healers on their curative effect in the treatment of malaria or sleeping sickness. The plant extracts were administered orally at doses rate of 100, 250 and 500mg/kg body weight for both methanolic and chloroform extract. *Tinospora bakis* extract was found to be effective in clearing the parasite for a considerate time and extending the life span of the experimental animal used. *Argemone maxicana* on the other hand was effective in reducing the parasiraemia. *Argemone Mexicana* on the other hand was found to be more effective in clearing or reducing the parasitaemia for both methanolic and chloroformic extract. *Aristolochia bracteolate*, chloroform extract gave a very good trypanocidal effect where clearance of the parasite was 100% where as the methanolic extract gave a limited trypanocidal effect (Samia, 2011).

Adeiza *et al.* (2009) reported on the evaluation of some medicinal plants for their in vitro activities on trypanosoma evansi. The results revealed that *Khaya senegalensis* and *Anonna senegalensis* immobilized the parasites at 10 mg/ml. Phytochemical profile of the plants

showed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides. The results obtained with these crude extracts showed that these plants are potential sources of trypanocidal drugs/chemical leads. Also the *in vitro* activities of the aqueous and ethanolic extracts of the leaves, root bark and stem bark of *Khaya senegalensis* on *Trypanosoma evansi* have been evaluated (Umar *et al.*, 2010) with minimum inhibitory concentration of 1 mg/ml, for the *in vivo* studies the extract significantly kept the parasitemia lower than was observed in the control. All these works has proven the efficacy of plants used in the treatment of trypanosomosis in northern Nigeria by traditional healers and Fulani herds men who have been known to keep lives stock.

The active principle responsible for the antitrypanosomal effects in some plants has been reported. Results on the *in vivo* and *in vitro* activities of medical plants on haemic and humoral trypanosomes revealed that several medicinal plants by researchers were based on their trypanocidal claims as documented in ancient pharmacopoeia, knowledge from traditional healers, herdsmen, village elders and feeding habits of large primates. The plants were subjected to various methods of extraction. The choice of extraction method depended largely on the part of the plant to be tested and often, fractionated through thin layer chromatography, infrared spectroscopy to yield bioactive components. This was with a view of elucidating structural components and possible synthesis of new trypanocide. The commonly encountered active principles in the extracts were saponins, terpenes, phenolics, flavonoids, tannins, glycosides, anthraquinones, columbines, neolignan, quinines, phlobatanin, resins and alkaloids. These fractions produced efficacy either singly or synergistically at dosages (<800mg/kg) *in vivo*, leading to the elimination of parasitaemia, modulating decline red cell indices and the

alleviation of clinical signs of trypanosomosis. Most of the extracts however, produced effect *in vitro* within minutes of application in a graded dose manner. The extracts in most cases produced signs of acute toxicity (*in vivo*) at dosages (<800mg/kg) leading to degenerative changes in vital organs. Signs of cytotoxicity were also encountered *in vitro* on various cell lines. Therefore the folkloric medicinal applications of plants for the treatment of trypanosomosis have a pharmacological basis (Mbaya and Ibrahim, 2011).

Some of the natural product with antitrypanosomal activity includes; Alkaloid, isolated from plants and marine organisms have been tested on trypanosomes, some showed appreciable activity both *in vitro* and *in vivo* while others showed activity only *in vitro*. Several alkaloids have been tested on trypanosomes *in vitro*. In a study of 34 different alkaloids, those with a piperidine, pyridine, tropane, quinolizidine, indole, or purine skeleton were found, in general, to be inactive on *T. b. brucei* and *T. congolense* bloodstream forms (Merschjohann *et al.*, 2001). Several alkaloids isolated from marine organisms such as sponges, ascidians and tunicates have been evaluated for their antitrypanosomal activity. Lepadins isolated from a tunicate species from the genus *Didemnum*, possess an unusual decahydroquinoline skeleton and show significant and selective antitrypanosomal activity *in vitro*. The quinoline alkaloids from *Cinchona* bark (*Rubiaceae*) (quinidine, cinchonine, quinine, cinchonidine) had significant trypanocidal activity with IC₅₀ values of 0.8, 1.2, 4.9, 7.1 μM, respectively, on *T. b. brucei* (Merschjohann *et al.*, 2001).

Phenolic derivatives for e.g. Ascofuranone, as shown in fig 2.3, a prenylated phenol antibiotic isolated from a phytopathogenic fungus, *Ascochyta visiae* had a potential antitrypanosomal

activity both *in vitro* and *in vivo* though when combined with glycerol (Minagawa *et al.*, 1997). Other simple phenolic compounds which are widely distributed in plants have been tested for their antitrypanosomal activity as well. Gallic acid, a well-known component of hydrolysable tannins, is equally active on the bloodstream and procyclic forms of *T. b. brucei* ($IC_{50} = 15.6$ and $14.4 \mu\text{M}$ respectively) (Koide *et al.*, 1998).

Bio-guided fractionation of a stem bark extract of *Combretum molle* (Combretaceae) led to the isolation of two classes of compounds: two inactive saponins and two hydrolysable tannins, punicalagin as shown in fig 2.4, and a structurally similar compound for which the full structure has not yet been elucidated (CM-A). Those two compounds exhibited activity on the mammalian stage of *T. b. rhodesiense* ($IC_{50} = 1.75$ and $1.5 \mu\text{M}$ for punicalagin and CM-A). These two tannins showed a relative selectivity against trypanosomes, a result which contradicts the general belief that tannins are non-selective enzyme inhibitors due to their substantial number of phenolic groups (Asres *et al.*, 2001).

The medicinal plant *Brucea javanica* (L) Merr. (Simaroubaceae) is widely distributed throughout Asia where its bitter fruits have been used in traditional medicine for various ailments. Fifteen C-20 quassinoids were isolated from the fruits of *B. javanica* and examined for their *in vitro* antitrypanosomal activities against trypomastigotes of *Trypanosoma evansi*. Bruceine A, bruceantinol, bruceine C, brusatol, and bruceine B showed strong antitrypanosomal activities with IC_{50} values in the range of 2.9–17.8 nM, which compared well with the standard trypanocidal drugs diminazene aceturate ($IC_{50} = 8.8 \text{ nM}$) and suramin ($IC_{50} = 43.2 \text{ nM}$). However, dehydrobruceine A, dehydrobruceine B, and dehydrobrusatol were

about 2100, 900, and 1200 times less active, respectively, than bruceine A, bruceine B, and brusatol. The relationship of the structure and antitrypanosomal activity of these quassinoid compounds suggested that the presence of a diosphenol moiety is important for their activities against *T. evansi* (Saw et al 2008).

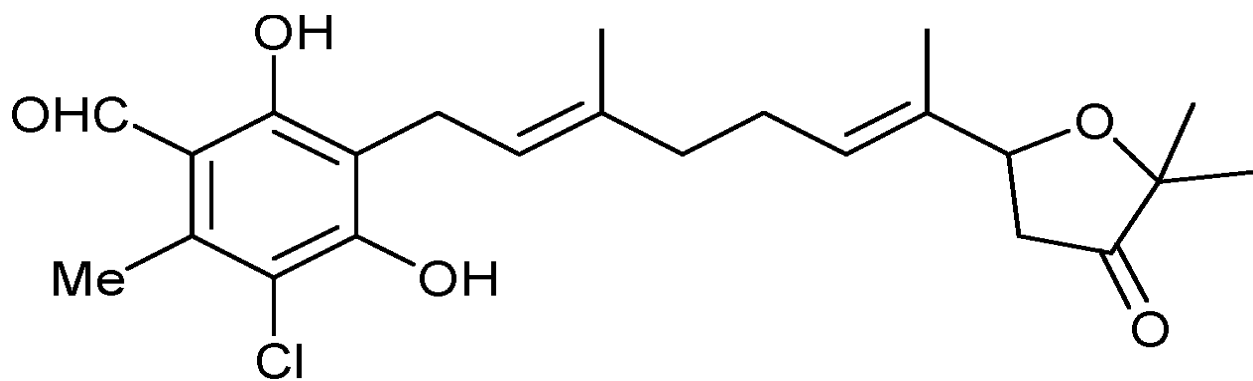
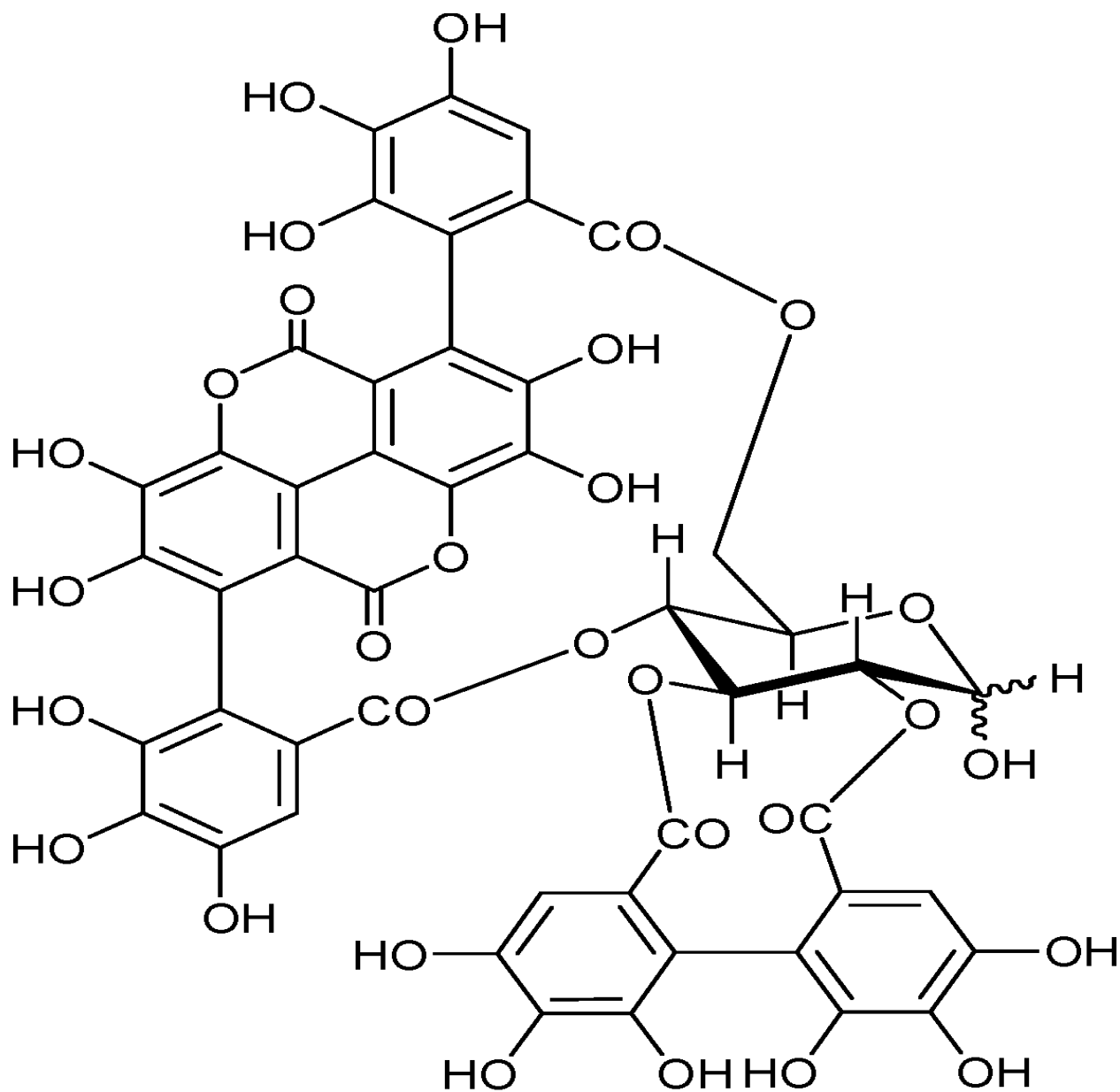


Fig 2.3 Ascofuranone

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353



55

Fig 2.4 Punicalagine

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

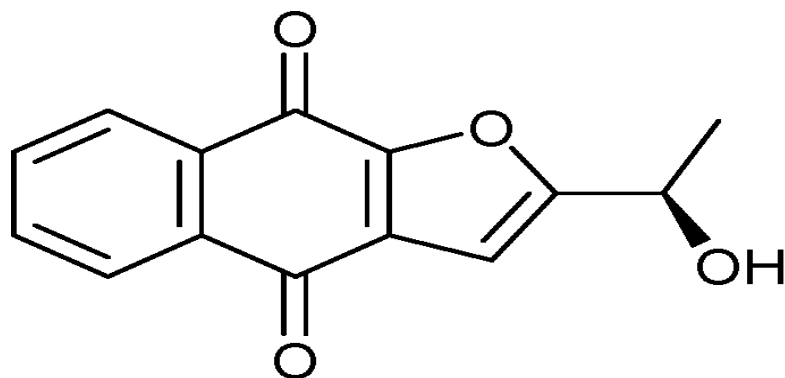


Fig 2.5 (1-hydroxyethyl)-naphtho-[2,3-*b*]furan-4,9-quinone.

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

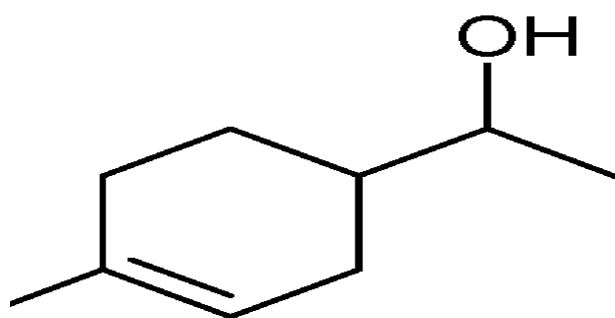


Fig 2.6. Terpinen-4-ol.

Hoet S., Opperadoes F., Brun R.,and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

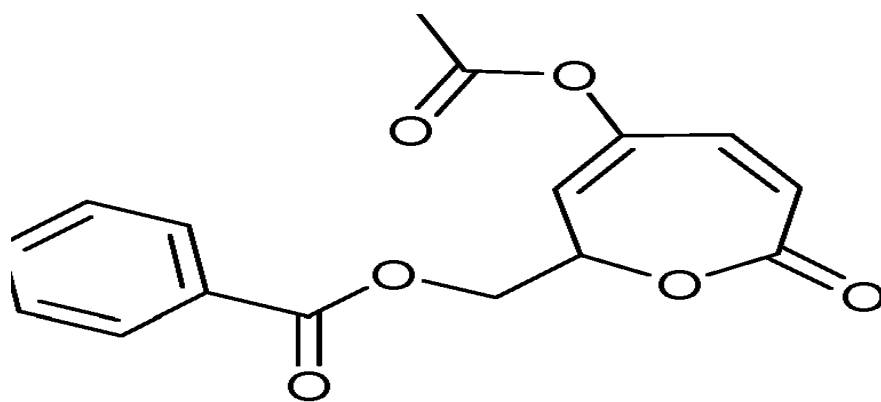


Fig 2.7 (5-acetoxy-7-benzoyloxymethyl-7H-oxepin-2-one)

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

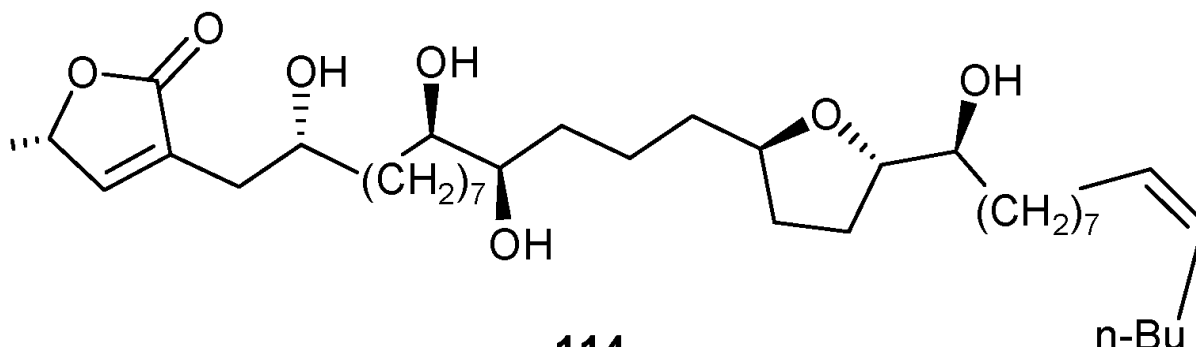


Fig 2.8 Senegalene

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

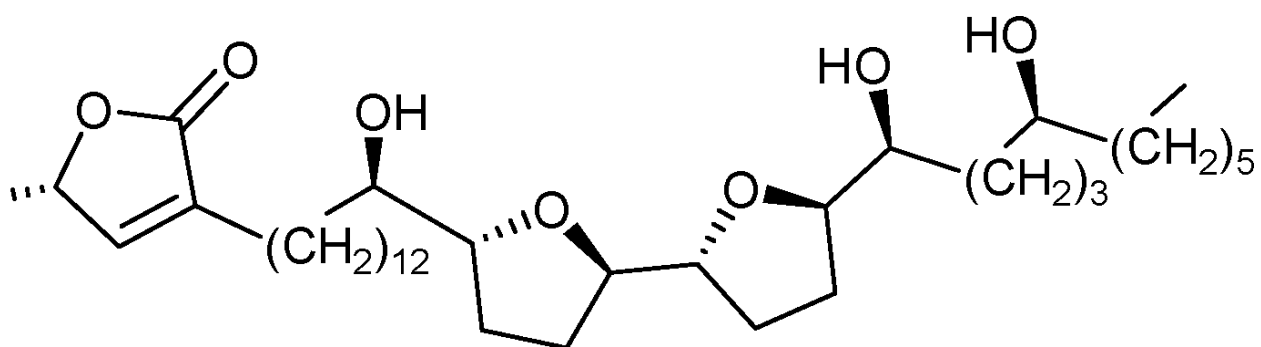


Fig 2.9 Squamocine

Hoet S., Opperadoes F., Brun R., and Quetinleclereq, (2004a) Natural products active against

African trypanosomes : a step towards new drugs. *Natural product*, 21:353

Four methoxylated flavones were isolated by bio-guided fractionation from the leaves of *Ehretia amoena* (Boraginaceae), a plant traditionally used in Uganda to treat sleeping sickness: chrysosplenetin, chrysosplenol, retusin and artemetin (Raz, 1998). Chrysosplenetin and chrysosplenol were the most potent on *T. b. rhodesiense* bloodstream trypomastigotes (IC_{50} = 2.9 and 4.7 μ M, respectively) while retusin was less active (IC_{50} =14.2 μ M) although, artemetin was inactive. Several diarylheptanoids isolated from species of the Zingiberaceae family have been tested for their antitrypanosomal activity (Nose *et al.*, 1998 and Kamanaing *et al.*, 2003). Curcumin, isolated from the rhizomes of *Curcuma longa*, displayed IC_{50} values of 0.83 μ M and 4.35 μ M for the bloodstream and procyclic forms of *T. b. brucei*, respectively.

Quinones have been evaluated for their antitrypanosomal activity for example Diospyrin ,a bis-naphthoquinone isolated from the bark of *Diospyros montana* (Ebenaceae), as well as semi-synthetic derivatives, have been investigated for their antitrypanosomal activity *in vitro* on *T. b. brucei* bloodstream forms. The IC_{50} of diospyrin was 50 μ M (Yardley *et al.*, 1996). Activity-guided fractionation of stem bark and root bark extracts of *Kigelia pinnata* (Bignoniaceae) allowed the isolation of one furanonaphthoquinone, 2-(1-hydroxyethyl)-naphtho-[2,3-*b*]furan-

4,9-quinone (Fig 2.5), which possessed a pronounced activity against both *T. b. brucei* and *T. b. rhodesiense* bloodstream forms with $IC_{50} = 0.12 \mu\text{M}$ and $0.045 \mu\text{M}$ respectively.

Mikus *et al* (2000) evaluated the effect of several mono- and sesquiterpenes, which are frequently present in essential oils, on the viability of *T. b. brucei* bloodstream forms and of human HL-60 cells. The only active monoterpene was terpinen-4-ol, (Fig 2.6) a monoterpene alcohol, which exhibited a high antitrypanosomal activity ($IC_{50} = 0.13 \mu\text{M}$)

Several terpenoids, as well as a coumarin, were isolated from the leaves of *Guarea rhopalocarpa* (Meliaceae): only two lanostane triterpenoids showed some effect against the mammalian stage of *T. b. brucei*. 23-Hydroxy-5 α -lanosta-7,9(11), 24-triene- 3-one was active with an IC_{50} of $5 \mu\text{M}$. 5 α -Lanosta- 7, 9(11),24-triene-3 α ,23-diol, with a hydroxyl group on C3 instead of a ketone had an enhanced activity ($IC_{50} = 1.75 \mu\text{M}$) and was 12 times less cytotoxic to KB cells ($IC_{50} = 21.2 \mu\text{M}$). Various terpenoids were also isolated from the leaves of *Celaenodendron mexicanum* (Euphorbiaceae) but only one showed activity against *T. b. brucei* bloodstream forms: 3-oxotirucalla- 7,24Z-dien-26-oic acid, a tirucalla-type triterpene, had IC_{50} values of $16.8 \mu\text{M}$ and $137.6 \mu\text{M}$ on trypanosomes and KB cells respectively (Camacho *et al.*, 2000).

Other compounds can also be responsible for antitrypanosomal activity and examples include as obtained from Bio-guided-fractionation of a methylene chloride extract of the stems of *Uvaria klaineana* (Annonaceae) which led to the isolation of klaivanolide (5-acetoxy-7-benzoyloxymethyl-7H-oxepin-2-one) as shown in Fig 2.7, a bisunsaturated seven-membered lactone which has a moderate activity on *T. b. brucei* bloodstream forms ($IC_{100} = 33.2 \mu\text{M}$) (Akendegue *et al.*, 2002). Acetogenins, such as senegalene and squamocine, isolated from the

seeds of *Annon senegalensis* (Annonaceae) show activity against bloodstream forms of *T. b. brucei* (IC₁₀₀ = 16 µM)

2.7 EUPHOBIACEAE

The family (Euphorbiaceae) is a large family of flowering plants with 300 genera and around 7,500 species that are further characterized by the frequent occurrence of milky sap. The leaves are mostly alternate but may be opposite or whorled and they are simple, or compound, or sometimes highly reduced. Stipules are generally present but may be reduced to hairs, glands or spines. The flowers are unisexual and usually actinomorphic. They may be highly reduced by suppression of parts, in the extreme form consisting of a naked stamen as a male flower and a naked pistil as a female flower (Charles *et al.*, 2007). Most are herbs, but some, especially in the tropics, are also shrubs or trees. Some are succulent and resemble cacti. This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. There is a large variety in tropical Africa, but it is not as abundant or varied as in these two other tropical regions (Betancur-Galvis *et al.*, 2002). Among the genera that belongs to this family *Hymenocardia* is one of them. *Hymenocardia* has upto 17 to 21 recorded species but the ones alive are *Hymenocardia acida* and *Hymenocardia ulomoides* (Burkhill, 1994)

2.8 HYMENOCARDIA ACIDA

2.8.1 Taxonomy

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Malpighiales*

Family: *Euphorbiaceae*

Genus: *Hymenocardia*

Specific epithet: *acida* - Tul.

2.8.2 Common Name

Large red-heart

2.8.3 Botanical Name

Hymenocardia acida Tul.

(Bisby *et al.*, 2008)

2.8.4 Local Names

Yoruba: Orupa

Hausa: Jan yaaro

Igbo: Ikalanga

Fula-fulfude: Booehi

(Burkhill 1985)

2.8.5 Botanical Distribution

Hymenocardia acida is a small savannah tree or shrub about 6 m high gnarled and twisted with characteristic rough rusty-red bark, of the wooded savanna throughout the Region from Senegal to Cameroon, and widespread in tropical Africa. The wood is light brown or pink, darkening to orange, close-grained, with conspicuous annual rings, and hard (Burkhill, 1985). It has been said to be brittle and good only for firewood. The leaves are thin, leathery, elliptic-oblong up to 8.75 cm long and 3.75 cm broad, apex obtuse to rounded, base obtuse; petiole slender, up to

1.8 cm long. Leaves are usually pubescent when young with a dense mat of fine hairs and with golden glands beneath. Flowers are unisexual, male flowers reddish-yellow occurring in clusters of spikes up to 6.5 cm long; calyx cupular, red, anthers creamy white . Female flowers green, placed on axils of leafy lateral branches and bearing a prominent crimson stigma spreading about 1.25 cm. The fruit are compressed, obcordate and reddish-brown, 2.5 cm long and 2.5-3.75 cm broad and developing in pairs along one edge, each with a thin pale brown nearly square wing. Seed are flattened and glossy brown. The generic name *Hymenocardia* is derived from the Greek words ‘hymen’ - membrane and ‘kardia ’- heart, in reference to the heart-shaped fruits which have a transparent covering membrane (hymen). The specific epithet *acida* describes the sour taste of its fruits (Burkhill, 1994).



Plate 2.10 *Hymenocardia acida* plant.

Hyde, M. A, Wursten, B. T and Ballings P. (2012) Flora of Zimbabwe: Species information individual images; *Hymenocardia acida*



Plate 2.2 Stem bark of *Hymenocardia acida*

2.8.6 Ecology and Distribution

2.8.6.1 *Natural Habitat*

H. acida is commonly found in savannah, scrub and open woodland in association with *Parinari curatellifolia*, *Isobertia*, *Stereospermum kunthianum*, *Parkia clappertoniana* and *Protea madiensis*. Dry seasons last long in its natural range (Burkhill, 1994).

2.8.6.2 *Geographic Distribution*

Native : Angola, Cameroon, Chad, Congo, Cote d'Ivoire, Gambia, Ghana, Guinea-Bissau, Kenya, Mali, Mozambique, Niger, Nigeria, Senegal, Tanzania, Togo, Uganda, Zambia, Zimbabwe (Burkhill, 1994).

2.8.6.3 *Biophysical Limits*

Altitude: 500-1 200 m Soil type: Can grow on sandy, loam and clayey soils (Burkhill, 1994).

2.8.6.4 *Reproductive Biology*

H. acida is dioecious, male and female flowers occurring on different trees. Flowering starts from September-November. Seeds mature from June-September (Burkhill, 1994).

2.8.7 Propagation and Management

2.8.7.1 *Propagation Methods*

Direct seeding is the most reliable propagation method (Burkhill, 1994).

2.8.8 Functional Uses

2.8.8.1 *Products*

Leaves and stem bark and roots are commonly used as medicine. A leaf infusion is taken to treat chest complaints and smallpox, a leaf and root infusion is taken to treat oedema caused by malnutrition and an extract of the leafy twig is rubbed in to strengthen sickly children. A leaf concoction is taken to treat stomach ache, trypanosomosis and cough (Gabiella, 1996). Leaf sap is used as eye drops to treat eye infection, together with honey; it is used to treat gallbladder problems and fever, also as bath to treat fever and hemorrhoid. Leaf sap is also used as ear drops to treat otitis. Leaf powder is taken as snuff to treat headache. Leaf powder is tropically applied in friction to treat rheumatic pains, toothache and fever. It is also sprinkled on sores after washing. Leaf powder is taken in food to treat asthma. A leaf decoction is taken to treat snake bites. A decoction of the leafy twig is used for bathing to treat tetanus convulsion and exhaustion. The bitter stem bark is slightly astringent and causes copious salivation when chewed. In West Africa it is chewed with kola (*cola spp*) to treat dysentery. A bark concoction is widely taken to treat pulmonary infection including tuberculosis. It is also used as a stem bath alone or with the fruits to treat breathing difficulties and colds. The powdered bark together with parts of other plants is used as a macerator to treat fractures (Gabiella, 1996).

The powdered bark with copper dust is sprinkled on syphilitic sores. Powderd bark in water or pulped bark is taken internally to treat abdominal pains, diarrhea, dysenter, menstrual pains female infertility painful swellings coughs and epileptic fits. The powdered bark concuction is applied as poultice to treat colic abscessors and tumour eye infection, migraine and also skin infection such as itch prickly parasites and leprosy (Burkhill, 1985). Powdered root in porridge is given to breast-feeding women to diminish the milk flow. In central Africa the brownish red dye obtained from the bark is used to colour raffia work and cloth. The bark contains much

tannins and it's used for tanning leather. The wood is commonly used as fuel for charcoal production it burns slowly with hot flame and little smoke. It is planted for erosion control leafy shoot are sometimes eaten as condiment and the twigs are used as tooth brush. The stem bark is made into rope (Kokwaro, 1976).

2.8.8.2 Properties

Preliminary studies of the chemistry showed presence of saponnins. Stem bark contains triterpenoids which are associated with its antimalarial and anti-inflammatory activities. The stem barks of the plant *Hymenocardia acida* (Tul) has been reported to yield five triterpenoids which were identified by spectroscopic methods as friedelan-3-one, betulinic acid, lupeol, _-sitosterol, stigmasterol and the fatty acid, oleic acid (Igoli, 2008).

A dichloromethane extract of the leafy twigs showed significant antitrypanosomal activity. Methylene chloride and methanolic stem bark and root extracts showed moderate activity against plasmodium falciparum in vitro (Vonthron-senecheau *et al.*, 2003). Crude root extract showed significant antihelminthic activity against intestinal parasite. Ethanolic extract of the leaf showed significant effect against sickle cell formation (Ibrahim *et al.*, 2006) also an ethanolic root extract showed marked antibacterial activity against klebsellia pneumonia, staphylococcus aureus, streptococcus and salmonella (Hoet *et al.*, 1994b). It showed anti-inflammatory activities in vitro in rats and mice. An aqueous stem bark extract showed significant antiulcer activity against induced gastric lesions in rats. A methanolic leaf extract showed very high radical scavenging activity compared to tocopherol.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant Collection:

The plant was collected from Ahmadu Bello University, Zaria environment, then identified and authenticated at the Herbarium in Biological Sciences Department, Ahmadu Bello University, Zaria.

3.1.2 Reagents

Chemicals and reagents were obtained from sigma chemical company St Louis, USA, Zaf pharmaceuticals PLC Addis Ababa, Ethiopia. Shanghai Veterinary Pharmaceutical, 500 xin Bei Road Shangai, Chaina and they include:

- (i) Petroleum ether
- (ii) Chloroform
- (iii) Methanol
- (iv) Dextrose saline
- (v) Phosphate Buffer Saline Glucose
- (vi) Heparine
- (vii) Normal Saline

(viii) Diminaveto[®]

(ix) Glucose

(x) Silica gel

(xi) Disinfectants

(xii) Ethyl acetate

3.1.3 Equipment

(i) Soxhlet Extractor

(ii) Rotary Evaporator (Model No:262803)

(iii) Microscope (Model No: 32695-29)

(iv) Chromatographic Tank

(v) Vacuum Liquid Chromatography apparatus

(vi) Pre coated Thin Layer Chromatographic plates

3.1.4 Instrumentation

(i) Gas Chromatography-Mass Spectroscopy (GCMS)

Agilent Gas Chromatogram (6890N model) coupled to 5973 Mass Selective Detector was used.

(ii) Ultraviolet lamp

3.1.5 Animals

Mature albino rats free from infection was purchased from National Research Institute for Chemical Technology, Basawa, Zaria and kept with food and water to acclimatise for 2 weeks.

3.1.6 Parasites

Trypanosoma evansi parasites were obtained from the Department of Veterinary Parasitology, Ahmadu Bello University, Zaria. White albino rats were infected with *Trypanosoma evansi* parasites by intraperitoneal inoculation.

3.2 METHODS

3.2.1 Determination of Parasitaemia

The parasites were maintained in the laboratory by continuous passage in rats until it was required. The passage was with infected blood containing 1×10^3 parasites per field of view and was introduced intraperitoneally into healthy rats in 0.1–0.2ml blood/physiological saline solution. The number of parasites was determined microscopically at magnification of $\times 400$ and parasitaemia was monitored daily in the inoculated rat by obtaining blood daily from pre-sterilized tail (Herbert and Lumsden, 1976).

3.2.2 Sample Preparation and Extraction

Stem bark of the plant which was collected, air dried at room temperature (28°C-30°C) was pulverized and stored in air-tight polythene bags until required. The plant material (1.5kg) was extracted with various solvents; petroleum ether, chloroform and methanol (all distilled) using soxhlet extractor. The extract was then concentrated using a rotatory evaporator at 40 °C (Nataraja, 2003). Percentage recovery of the crude extracts was calculated using:

3.2.3. *In Vitro* Studies

The Drug Incubation Infectivity Test was carried out with wet blood films method (Chappuis *et al.*, 2005) to detect any motile trypanosomes and it was done in triplicates in a 96 well microtitre plate. Portions (10, 20, 40, 80 and 100mg/ml) of each extract was prepared and 100µl of infected blood was incubated with 25µl of each extract concentrations (previously prepared). Parasitaemia was monitored between 1–20 minutes of incubation at 30°C. About 0.5ml–1ml of test mixtures was observed every 1 minute under a microscope using ×400 powers. A set of Positive Control (PC) and Negative Control (NC) was set up to stand for 4hrs. The PC contained 25mg/ml of DIMINAVETO^R (1.05 g of Diminazene diacetate and 1.31gAntipyrine) and the NC contained only the infected blood suspended in heparin and Phosphate Buffer Saline Glucose (pH 7.2).

3.2.5 Phytochemical Screening

Chemical tests were carried out on methanolic extract to identify the secondary metabolites using standard procedures as described by Edeoga *et al* (2005).

- i. *Test for Glycosides:* Concentrated Sulphuric acid (5ml) was added to 0.25g of the extract and boiled for 15 minutes, this was then cooled and neutralized with 20% potassium hydroxide (KOH) the mixture was divided into two portions. Another 0.25g of the extract was also dissolved in distilled water this was used as a control.

- ii. *Test for Free Anthraquinones (Borntrager's test)*: Portions (0.25g) of the extract was shaken with 10ml of benzene and filtered. Portions of 5ml of 10% of ammonia solution was added to the filtrate and stirred, production of pink-red or violet colour to indicate the presence of free anthraquinones.
- iii. *Test for Cardiac Glycosides (Kella-Killiani test)* the extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degree, 1ml of concentrated sulphuric acid was added down the side and observed for purple ring colour at the interface.
- iv. *Test for Saponins* About 2g of the sample was boiled in 20ml of distilled water in a water bath and filtered. A 10ml portion of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.
- v. *Test for Steroids (salkowski reaction)* About 0.5g of extract was dissolved in 2ml of chloroform and a few drops of concentrated sulphuric acid was added to form a lower layer. A reddish colour observed at interface indicated presence of a steroidal ring.
- vi. *Test for Triterpenes (Lieberman-Burchards test)* Equal volume of acetic anhydride was added to the extract. 1ml of concentrated sulphuric acid was added down the side of the tube, the colour change was observed immediately and later ,red pink or ourple colour indicated the presence of triterpenes

- vii. *Test for Flavonoids* Dilute ammonia (5ml) was added to a portion of the plant extract followed by addition of concentrated sulphuric acid. A yellow colouration observed indicated the presence of flavonoides.
- viii. *Test for Tannins* About 0.5g of the extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.
- ix. *Test for Alkaloids (Meyer's test)* Few drops of meyer's reagent was added to a sample of the extract in a test tube, cream precipitate observed indicated presence of alkaloids.

3.2.6 Acute Toxicity Studies (LD₅₀)

The method of Lorke (1983) was used to investigate the immediate or acute toxicity in rats. It was carried out with a total of 15 rats randomly selected to acclimatise for one week. The experiment was divided into 2 phases. The first phase was divided into 3 groups of 3 rats each to which was administered intraperitoneally 10, 100 and 1000mg/kg/body weight of methanolic extract while the second phase divided into 3 groups of 2 rats each was administered intraperitoneally 200, 300 and 400mg/kg/body weight of methanolic extract. Each rat was monitored for a period of 48 hours for clinical signs and death.

3.2.7 *In Vivo* Studies

Animals were divided into 8 groups of 3 rats each and the groups were labeled A-H. Animals in groups A-G were infected intraperitoneally with 10⁴ T. evansi parasites. Animals in groups A-E were treated intraperitoneally with methanolic extract at 40, 60, 70, 80, 90 mg/kg/body weight/day and animals in group F were treated with Dimenaveto[®] (3.5mg/kg/body

weight/day), immediately after parasitaemia was detected (7 days post infection) and treatment was done for 10 consecutive days. Animals in group G was injected intraperitoneally with physiological saline and served as negative control (NC) and animals in groups H were uninfected and untreated. Parasitaemia was monitored daily while the packed cell volume was determined every 5 days (Abubakar *et al.*, 2009).

3.2.8 Prophylactic Studies

A group of 15 rats was divided into 5 groups. A, B, C received the extract at 90mg/kg/body weight for 1 day, 2 days and 3 days. Group D that served as negative control was not given any drug or extract. Members of groups A, B, C were challenged with parasite after 1 day, 2 days and 3 days respectively. Group E that served as positive control was treated with the drugs before infection.

3.2.9 Partial Isolation and Characterization of the Active Principle

3.2.9.1 *Thin-Layer Chromatography (TLC) Analysis and Choice of Mobile Phase*

The extract (methanol extract) used in the *in vitro* and *in vivo* studies, was partitioned in ethyl acetate and methanol to reduce complexity. This was done by first dissolving the methanol extracts in distilled water then it was transferred into 1000ml separatory funnel and 500ml ethyl acetate was used to shake it in a slanted manner. It was allowed to settle and the organic layer was collected. This was repeated about six times on the aqueous layer. The ethyl acetate extract collected was concentrated and allowed to dry.

TLC was used to determine the number of chemical components in the ethyl acetate extract and the most suitable mobile phase for good resolution. The ethyl acetate extract was subjected to thin layer chromatographic analysis to find the solvent system that will give better separation of the components therein. This was achieved using pre-coated TLC plates. A micro quantity of the sample solution was spotted on TLC pre-coated (MERCK) F254 plates, and developed with various ratios of organic solvents (petroleum spirit, ethyl acetate, methanol, chloroform) taking into consideration of their polarity. The solvent system that separated the components to high degree of resolution was considered. The plates were visualized under visible and UV-light (366 and 254 nm). The plates were sprayed with 10% sulphuric acid in methanol and heated at 100°C for 2-5 minutes (Touchstone, 1992). This detected any spot(s) that may not be detected by the UV lights. The solvent ratios that gave better resolution of spots were thereafter chosen as eluents for vacuum liquid chromatography. Appropriate fractions which were collected at intervals from the column were monitored by using TLC. These fractions that were obtained were subjected to *in vitro* and *in vivo* antitrypanosomal activity.

3.2.9.2 *Vacuum Liquid Chromatography*

The ethyl acetate extract was subjected to vacuum liquid chromatography (VLC) to fractionate the extract. Exactly 1.8g of ethyl acetate extract was dissolved in 20ml of ethyl acetate and pre-adsorbed on sephadex. The pre-adsorbed sample was allowed to dry and made into fine flowing powder using the rotatory evaporator under reduced pressure. The sintered glass funnel (porosity 3) used for the (VLC) was loaded with silica gel under vacuum ensuring that it was compacted and uniformly spread.

A non-polar solvent (petroleum ether) was run through the column under vacuum then the pre-adsorbed sample was evenly spread on the silica gel. Suction was applied to compress the sample to the silica gel and Whatman filter paper was used to cover the surface to prevent disturbance during the course of the experiment. The column was eluted with 1:1 ratio combination of ethyl acetate and petroleum ether and 33 fractions were collected (Aiyelaagbe *et al.*, 2007).



Plate 3.0 Vacum Liquid chromatography set up.

3.2.9 Spectroscopic Measurement

The fractions separated were analyzed using Agilent Gas Chromatography (6890N model) coupled to 5973 Mass Selective detector (MSD), in ethyl acetate as solvent. It was carried out in Faculty of Science, Usmanu Danfodio University Sokoto.

The results obtained were compared with an inbuilt main library (C:/Database/NIST02.L) and this library enabled the confirmation of the presence of some phytochemicals present in the plant.

3.2.10 Statistical Analysis

The experiments were carried out in triplicates. The results were presented as mean \pm standard deviation (SD). Student's t-test was used for comparison between the two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Characteristics of Petroleum ether, Chloroform and Methanolic Extracts of the Stem Bark of *Hymenocardia acida*.

Petroleum ether extract was greenish orange in colour and powdery. The chloroform extract was greenish in colour oily and sticky while the methanol extract was a shiny dark brown powder.

4.2 Percentage Yield of Extracts.

After the extraction the percentage yield calculated is given as shown in Table 4.1. in which methanolic extract gave the highest yield.

4.3 Parasitaemia in Rat Infected with *T. evansi*

After infection of rats with the *T. evansi*, clinical symptoms of trypanosomosis developed after six days. The clinical symptoms included acute bouts of fever, dullness, anaemia and high peaks of parasitaemia leading to death after 7-10 days.

4.4 *In vitro* Activity of Crude Extracts of the Stem Bark of *Hymenocardia acida*.

Table 4.2 shows the *In vitro* effect of petroleum ether, chloroform, and methanol extract of the stem bark of *H. acida* on *Trypanosoma evansi* at concentrations of 100, 80, 40, 20 and 10mg/ml. Petroleum ether extract had no noticeable effect on the parasite *in vitro* while chloroform extract had moderate activity, at 10mg/ml it cleared the parasite in less than 13 minutes, at 20mg/ml it cleared the parasite in less than 10 minutes and at 40mg/ml it cleared the

parasite in less than 9 minutes, at 80mg/ml it cleared the parasite in less than 4 minutes while at 100mg/ml it was able to clear the parasite in less than 2 minutes. Methanolic extract was the most active extract. This is because at almost all concentrations (20, 40, 80, 100mg/ml) it was able to clear the parasite in less than 1 minute except at 10mg/ml where it cleared the parasite in less than 3 minutes.

4.5. LD₅₀ of Methanolic Extract of the Stem Bark of *Hymenocardia acida*.

The Lethal Dose of the extract was calculated based on the result of acute toxicity observed in Tables 4.4 and 4.6. In the phase 1, all the animals administered 1000mg/kg/body weight died but those administered 100mg/kg/body weight and 10mg/kg/body weight survived. In the phase 2 all animals administered 300 and 400mg/kg/body weight died while those administered 200mg/kg/body weight survived. The LD₅₀ was calculated to be 245mg/kg/body weight.

Table 4.1 Percentage yield of the different solvent extraction on the stem bark of
Hymenocardia acida

Solvent Used	Yield (%)
Petroleum ether	0.5
Chloroform	0.3
Methanol	16

Table 4.2 *In vitro* effect of petroleum ether, chloroform, and methanol extract of the stem bark of *H. acida* on *Trypanosoma evansi*.

Concentration(mg/ml)	petroleum ether extract time (min)	chloroform extract time (min)	methanol extract time (min)
100	+	<2*	<1*
80	+	<4*	<1*
40	+	<9*	<1*
20	+	<10*	<1*
10	+	<13*	<3*

+ Presence of motility

* Absence of motility.

< Less than

Positive control: Standard drug used was Dimenazine aceturate at 3.5mg/ml it cleared the parasite completely

Negative control: The parasitaemia was present massively for 20 minutes in which the experiment lasted

Table 4.3a LD₅₀ of methanolic extract of stem bark of *Hymenocardia acida* (Phase 1)

Doses given(mg/kg)	Number of rats	Mortality
10	3	0/3
100	3	0/3
1000	3	3/3

Table 4.3b LD₅₀ of methanolic extract of stem bark of *Hymenocardia acida* (Phase 2)

Doses given (mg/kg/body weight)	Number of rats	Mortality
200	2	0/2
300	2	2/2
400	2	2/2

LD₅₀ = 245mg/kg/body weight

Table 4.4 Presence of secondary metabolites in methanolic extract.

Secondary Metabolites	Availability
Glycosides	Present
Anthraquinone	Absent
Cardiacglycosides	Present
Saponins	Present
Steroids	Present
Triterpenes	Present
Flavonoids	Present
Tanins	Present
Alkaloids	Present

4.7 In vivo Antitrypanosomal Activity of Methanolic Extract of *Hymenocardia acida* on *T. evansi*

Fig 4.1 shows that animals treated with 40mg/kg/bodyweight of crude methanolic extract survived for only 11 days which is almost the same with the positive control where the rats survived for 10 days. At this concentration, there was no noticeable effect of the extract on the rats. The survival days of the rats increased to 8 days compared to the negative control in fact, the motility of the parasite also reduced drastically. The survival days and the reduction in parasite motility increased as the concentration of the crude methanolic extract used for treatment increased, therefore at 90mg/kg/bodyweight the rats in this group had an increased survival days.

In Table 4.5 the Packed Cell Volume (PCV) shows that there was reduction in all infected rats treated with methanolic extract at all the concentrations compared to the positive control group whose PCV increased steadily. Those treated with the standard drug showed noticeable increase in PCV when compare with negative control group. There was also reduction of the PCV in rats used as negative control as the experiment progressed.

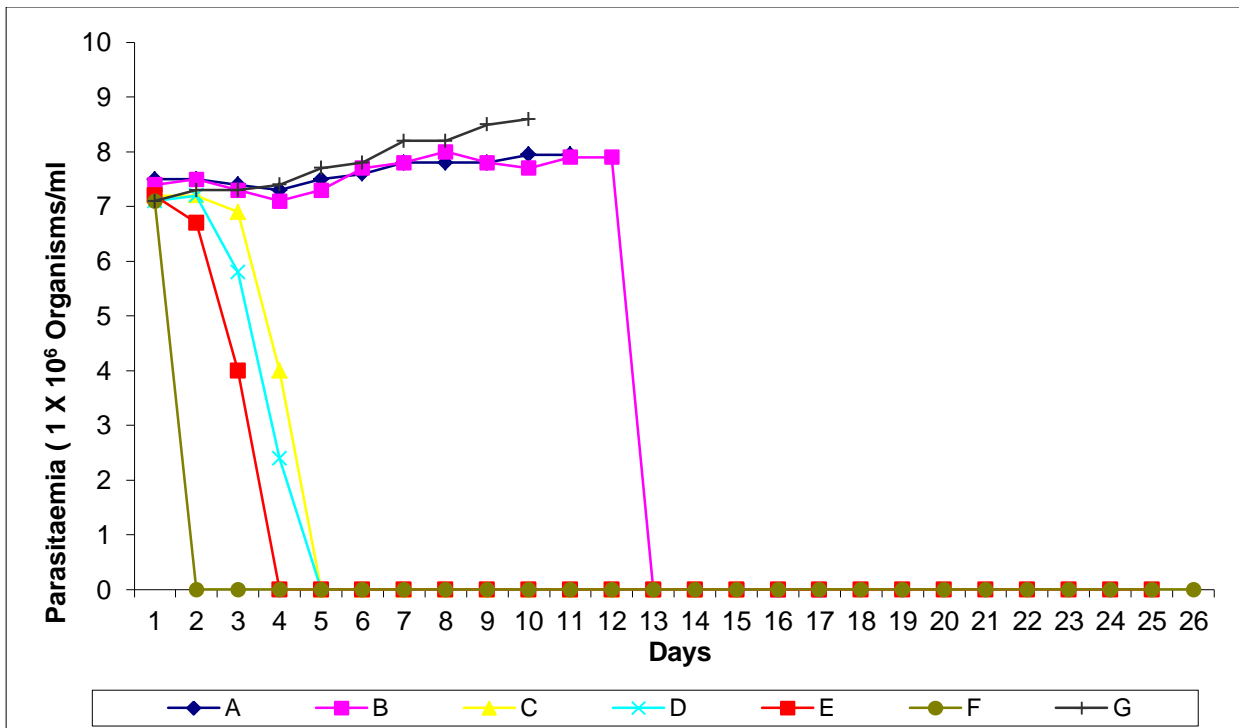


Fig 4.1 Parasitaemia level of *T. evansi* infected rats treated with crude methanolic extracts of *Hymenocardia acida* in vivo

A= 40mg/kg/body weight

B = 60mg/kg/body weight

C = 70mg/kg/body weight

D = 80mg/kg/body weight

E = 90mg/kg/body weight

F = standard drug (Dimenaveto®)

G = Infected and untreated (Negative control)

Table 4.5 Packed Cell Volume (PCV) values of animals infected with *T. evansi* and treated with crude extract of *Hymenocardia acida*

Groups	Day 1	Day 7	Day 12	Day 17	Day 21
A	51.67 ^{bc} ±0.88	40.67 ^a ±0.58		-	-
B	47.67 ^b ±1.86	43.33 ^{ab} ±1.20	39.33 ^{ab} ±0.33	36.50 ^a ±1.50	
C	52.33 ^c ±1.45	45.33 ^{bc} ±0.88	42.67 ^{ab} ±0.88		-
D	42.33 ^a ±1.20	46.33 ^{bc} ±1.45	38.67 ^a ±0.88		-
E	51.00 ^{bc} ±0.58	47.00 ^{bc} ±0.67	43.00 ^b ±1.00	38.33 ^a ±0.88	34.67 ^a ±1.53
F	48.00 ^{bc} ±2.31	48.67 ^c ±2.03	50.33 ^c ±2.04	51.33 ^b ±1.86	52.67 ^b ±3.06
H	41.33 ^a ±0.88	40.00 ^a ±0.58	42.00 ^{ab} ±0.57	47.00 ^b ±1.00	51.00 ^b ±5.77
G	38.33 ^a ±0.88	39.67 ^a ±1.53		-	-

Values with different superscripts along the same row are significantly different at $P \leq 0.05$

A = 40mg/kg/body weight

B = 60mg/kg/body weight

C = 70mg/kg/body weight

D = 80mg/kg/body weight

E = 90mg/kg/body weight

F = standard drug (Dimenaveto[®])

G = Infected and untreated

H = Uninfected and untreated

- = Dead

4.8 Prophylactic Effect of the Methanolic Extract at 90mg/kg/body weight

In the prophylactic studies, those animals in group A, (given 1 dose of the extract before infection) did not show any parasitaemia until the 5th day when there was a steady increase in parasitaemia until it died on the 11th day. Those animals in groups B (given 2 doses of the extract before infection) and C (given 3 doses of the extract before infection) did not show any sign of parasitaemia compared to the negative control which showed steady increase in parasitaemia right from the first day until it died on the 10th day.

4.9 Thin Layer Chromathography (TLC)

The weight of the extract gotten after partitioning was 2g and the colour was brown

The TLC was carried out on extract and the solvent mixture used was petroleum ether and ethyl acetate at ratio 1:1. This gave adequate separation of the component there in.

The R_f value for component 1 was 0.467 while that of component 2 was 0.17 after chromatographic studies.

Table 4.6 Prophylactic effect of *Hymenocardia acida* on *trypanosoma evansi* at 90mg/kg/body weight

Days	A	B	C	D	E
1	-	-	-	+1	-
2	-	-	-	+2	-
3	-	-	-	+2	-
4	-	-	-	+3	-
5	+1	-	-	+3	-
6	+3	-	-	+4	-
7	+4	-	-	+4	-
8	+4	-	-	+4	-
9	+5	-	-	+5	-
10	+5	-	-	D	-
11	D	-	-		-
12		-	-		-
13		-	-		-
14		-	-		-
15		-	-		-
16		-	-		-
17		-	-		-
18		-	-		-
19		-	-		-
20		-	-		-
21		-	-		-
22		-	-		-
23		-	-		-
24		-	-		-
25		-	-		-
26		-	-		-
27		-	-		-
28		-	-		-
29		-	-		-
30		-	-		-

+ 1 = Slightly motile, + 2 = Low motility, + 3 = Moderately motile, A= given methanolic extract of *H. acida* at 90mg/kg 1 dose, B = given methanolic extract of *H. acida* at 90mg/kg 2 doses, C = given methanolic extract of *H. acida* at 90mg/kg 3 doses, D = Infected and untreated, E = given standard drug (Dimenaveto[®]).

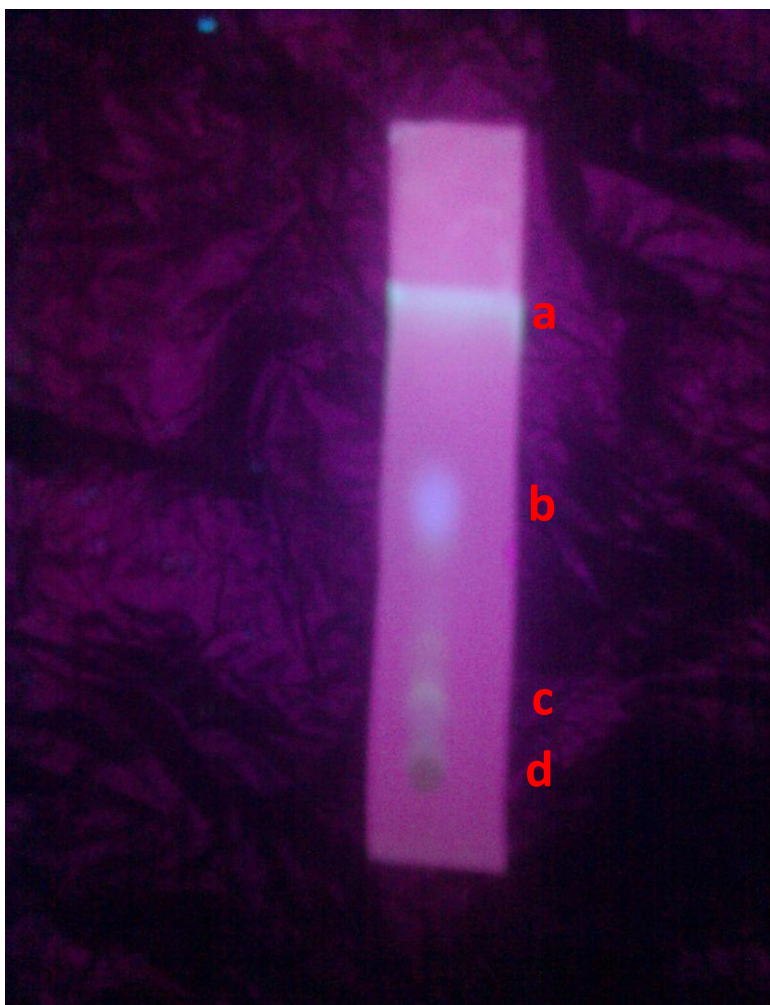


Plate 4.1 TLC of ethyl acetate extract using the solvent ethyl acetate and petroleum ether (1:1)

a = Solvent front	c = component 2
b = component 1	d = point of origin

4.10 Vacuum Liquid Chromatography

TLC of fractions (5-24) eluted by the solvent ethyl acetate and petroleum ether (1:1) had an R_f value of 0.486 which was pulled together and called fraction 1.

TLC of fractions 26-33 eluted by the solvent ethyl acetate and petroleum ether (1:1) had an R_f value of 0.17 which were pulled together and called fraction 2.

Fractions 1 and 2 were concentrated and dried, fraction 1, formed milk white crystalline powder which formed at the edges of the container which held them and was not soluble in the solvent (ethyl acetate to petroleum ether ratio 1:1) but was soluble in ethyl acetate and fraction 2, formed brown powder which was soluble in solvent (ethyl acetate to petroleum ether ratio 1:1) and ethyl acetate.

4.11 In vitro Antitrypanosomal Activity of Fraction 1 and 2

In vitro activity of fraction 1 and 2 showed different activities when exposed to the parasites under the same conditions. Table 4.15 shows the effect of these fractions on *T. evansi*

Fraction 2 showed more activity *in vitro* than fraction 1. At various concentrations, (5, 10, 20, 40, 80, 100mg/ml) fraction 1 was able to clear the parasite as concentration increased. At 80 and 100mg/ml it was able to clear the parasite in less than 1 minute. Fraction 2 was able to clear the parasite in less than one minute for all concentrations except at 10 and 5mg/ml where it was able to clear the parasite in less than 1 and 3 minutes.

4.12 In vivo Antitrypanosomal Activity of Fraction 1 and 2

The *in vivo* effect of the fractions on the extract showed that fractions 1 and 2 had the same effect on the parasites *in vivo* this is because fraction 1 cleared the parasites on day 4 while fraction 2 cleared the parasite on day 3 compared to the positive control where the parasite was present until the rat died on the 11th day. Survival days of the rats treated with the fractions increased by 19 days compared with the positive control.

In Table 4.9 the Packed Cell Volume (PCV) showed that there was reduction in the PCV of all infected rats treated with the fractions compared to the Negative control group whose PCV increased steadily. Those treated with the standard drug showed noticeable increase in the PCV also when compared with the negative control. As for the Positive control there was also reduction in the PCV of the rats as the experiment progressed.

Table 4.7. *In vitro* effect of fractions 1 and 2 on *T. evansi*.

Concentration(mg/ml)	Fraction 1 time (min)	Fraction 2 time (min)
100	<1*	<1*
80	<1*	<1*
40	<4*	<1*
20	<8*	<1*
10	<12*	<1*
5	<14*	<3*

+ Presence of motility

* Absence of motility.

< Less than

Positive control: Standard drug used was Dimenazine acetate at 3.5mg/ml it cleared the parasite completely

Negative control: The parasitaemia was present massively for 20 minutes in which the experiment lasted

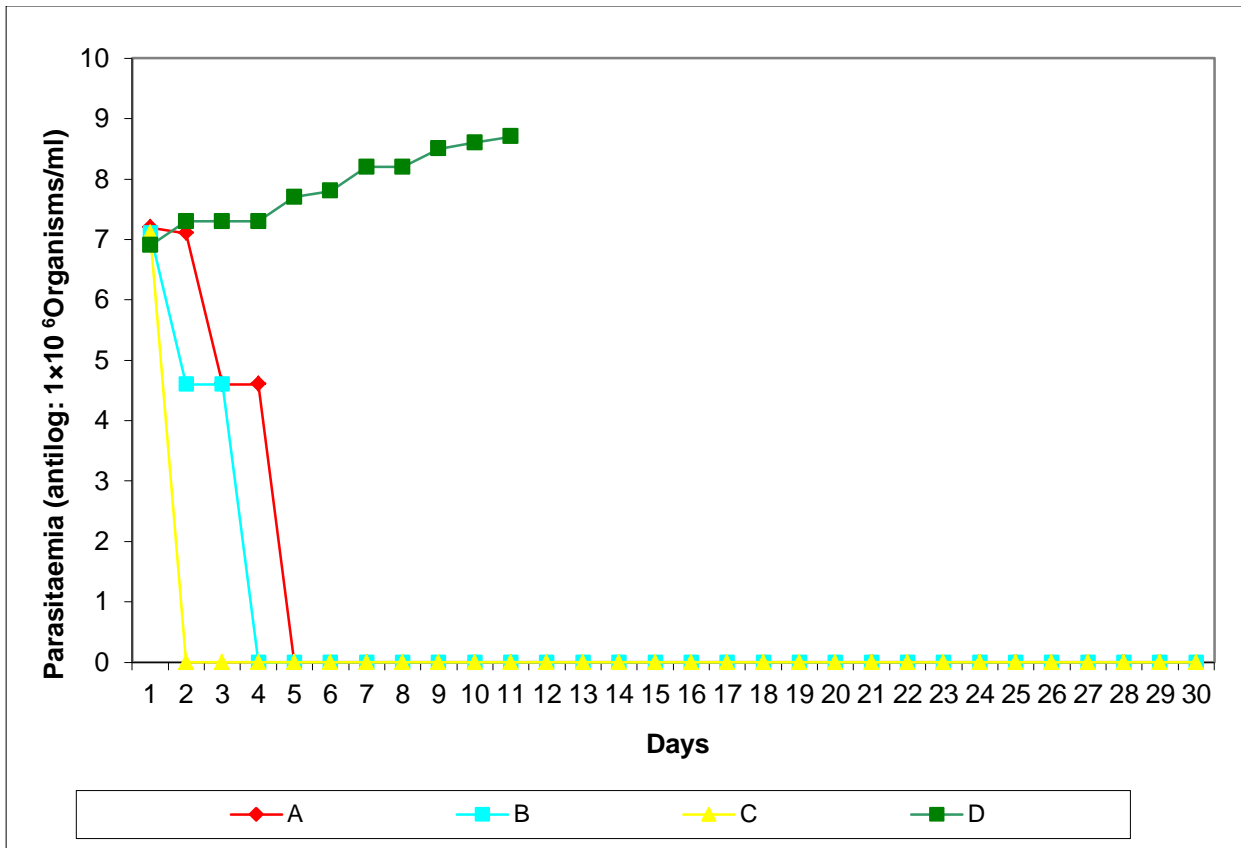


Fig 4.3 Parasitaemia level of *T. evansi* infected rats treated with fractions 1 and 2 of *Hymenocardia acida* in vivo

A= Fraction 1

B = Fraction 2

C = standard drug (Dimenaveto[®])

D = Infected and untreated (Negative control)

Table 4.8 Packed Cell Volume (PCV) values of animals infected with *T. evansi* and treated with fractions of *Hymenocardia acida*

Group	Day 1	Day 7	Day 12	Day 17	Day 21
A	37.00 ^b ±0.57	34.67 ^a ±0.88	33.00 ^a ±1.53	31.33 ^a ±1.20	29.33 ^a ±1.76
B	36.33 ^{ab} ±0.88	34.33 ^a ±0.88	32.33 ^a ±1.20	30.00 ^a ±1.00	26.33 ^a ±0.88
C	34.33 ^{ab} ±1.45	37.00 ^{ab} ±1.15	39.67 ^b ±0.88	41.00 ^b ±0.58	44.00 ^b ±0.58
D	33.67 ^a ±0.33	37.33 ^{ab} ±0.33	41.67 ^b ±0.88	46.33 ^c ±1.67	49.67 ^c ±1.20
E	41.33 ^c ±0.88	38.67 ^b ±1.20		-	-

Values with different superscripts along the same row are significantly different at $P \leq 0.05$

A = fraction 1

B = fraction 2

C = Standard drug (Dimenaveto[®])

D = Uninfected and untreated

E = Infected and untreated

- = Dead

4.13. Gas Chromatography-Mass Spectrograph (GC-MS) of the Fractions

When molecules are battered by electrons from the electron gun of a GC-MS machine, they receive such a thrump that the energy causes them to break up or fragment, there by producing a pattern of lines in the mass spectrum called a fragmentation pattern (Matthew, 2002).

Seven and six compounds were identified in fractions 1 and 2. These compounds were identified by GC-MS analysis, the active principle with their retention time, molecular weight, molecular formular and concentration% are presented in Tables 1 and 2. The prevailing compounds were; for fraction 1, Benzene Propanoic acid3,5-bis(1,1-dimethylethyl)-4-hydroxy-Octadecyl ester (34.02%), 1,3-Benzenediol,4-propyl- (31.29%), oxygenated hydrocarbon (13.82%) and 1,2-Benzenedicarboxylic acid,diisooctyl ester (11.10%). Others include 1,2-Benzenedicarboxylic acid,bis(2-Methylpropyl) ester (5.99%), and 1-Eicosene (03.78%) .

For fraction 2; 1,3-Benzenediol,4-propyl- (29.98%), 3',8,8'-Trimethoxy-3-Piperidyl-2,2'-binaphthalene-1,1',4',4'-tetrone (25.53%), Cholestan-3-one,cyclic1,2-ethanedieyl acetal(5.alpha)- (15.05%) and Octadecane,3-ethyl-5-(2-ethylbuthyl)- (10.2%). Others include 17-Pentatriacontene (09.94), Cyclohexane,1,1-dodecylidenebis[4-methyl-] (09.30%).

Table 4.9 Major Phyto-components obtained through the GC/MS study of fraction 1 in the ethyl acetate extract of *Hymenocardia acida*

No.	Retention Time	Name of Compound	Molecular Formular	Molecular Weight	Peak Area%
1	7.81	1,3-Benzenediol,4-propyl-	C ₉ H ₁₂ O ₂	152.19	31.29
2	14.47	1,2-Benzenedicarboxylic acid,bis(2-Methylpropyl) ester	C ₁₆ H ₂₂ O ₄	278.34	05.99
3	17.74	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.00	07.95
4	18.7	1-Eicosene	C ₂₀ H ₄₀	280.00	03.78
5	22.21	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.00	05.87
6	29.16	1,2-Benzenedicarboxylic acid,diisooctyl ester	C ₂₄ H ₃₈ O ₄	390.62	11.1
7	45.84	Benzene Propanoic acid3,5-bis(1,1-dimethylethyl)-4-hydroxy-Octadecyl ester	C ₃₅ H ₆₂ O ₃	530.88	34.02

Table 4.10 Major Phyto-components obtained through the GC/MS study of fraction 2 in the ethyl acetate extract of *Hymenocardia acida*

No.	Retention Time	Name of compound	Molecular Formular	Molecular weight	Peak area%
1	7.69	1,3-Benzenediol,4-propyl-	C ₉ H ₁₂ O ₂	152.19	29.98
2	29.14	3',8,8'-Trimethoxy-3-Piperidyl-2,2'-binaphthalene-1,1',4',4'-tetrone	C ₂₈ H ₂₅ O ₇	487.50	25.53
3	30.77	Octadecane,3-ethyl-5-(2-ethylbutyl)-	C ₃₅ H ₇₀	490.93	10.20
4	32.63	17-Pentatriacontene	C ₂₆ H ₅₄	366.00	09.94
5	36.78	Cholestan-3-one,cyclic1,2-ethanedieyl acetal(5.alpha)-	C ₂₉ H ₅₀ O ₂	430.7I	15.05
6	37.71	Cyclohexane,1,1-dodecylidenebis[4-methyl-]	C ₂₆ H ₅₀	362.68	09.30

CHAPTER FIVE

5.0 DISCUSSION

Natural products with antitrypanosomal activity and belonging to a variety of phytochemical classes have been identified. (Hopp *et al.*, 1976; Sepulveda-Boza *et al.*, 1996). The activity of the crude extracts of the plant *Hymenocardia acida* is in agreement with the findings of some other works who have shown that extracts of different plants may exhibit antitrypanosomal activity (Freiburghaus *et al.*, 1996; Atawodi *et al.*, 2003). The antitrypanosomal effect shown by these extracts might be attributed to the presence of some phytochemicals like flavonoids, terpenoids, tannins, alkaloids and steroids (Koide *et al.*, 1998; Asrer *et al.*, 2001). The *in vitro* and *in vivo* antitrypanosomal effect of the methanolic extract of *Hymenocardia acida* used in this study may be due to the presence of these phytochemicals.

Atawodi *et al* (2002) documented several plants which are used by Fulani herdsmen and other livestock farmers to treat trypanosomosis. In folkloric medicine of Idoma people of North Central Nigeria, *H. acida* is used alone or in combination to treat trypanosomosis and other fever related diseases (Ada & Claffey, 2003). Abu *et al.* (2009) showed that the extracts of *H. acida* stem bark exhibited trypanocidal activity. There have been previous reports of antitrypanosomal efficacy of the twig and leaf (Hoet *et al.*, 2004) and root bark (Atindehou *et al.*, 2004) extracts of *H. acida* of Beninese and Ivorian origins respectively, which is in line with this work.

Nsekuye *et al* (2006), in a similar study evaluated some medicinal plants from Mali for their *in vitro* and *in vivo* trypanosomal activity and established that out of 165 extracts of the plants screened for *in vitro* antitrypanosomal activity of which only 24 were active. This is in

agreement with the result of the evaluation of the petroleum ether, chloroform and methanol extracts in this study, where chloroform and methanol showed appreciable antitrypanosomal activity *in vitro*.

Also Umar *et al.* (2010) evaluated the *in vitro* and *in vivo* anti-*T. evansi* activity of the stem bark ethanolic extract of *Khaya senegalensis* which supports earlier reports that some plant extracts possess *in vivo* activities against trypanosomes (Nok *et al.*, 1993). Results of *in vitro* study in this work also showed that methanolic extract had the same highly appreciable effect on trypanosomes (*T. evansi*). In this study the *in vitro* effect of *H. acida* using petroleum ether, chloroform and methanol extracts against *T. evansi* showed that methanolic extract was the most active which made the focus of this work to be on the methanolic extract. The methanolic extract was able to clear the parasite in less than one minute at all concentrations used except at 10mg/ml in which the parasite was cleared in less than three minutes which suggests a dose dependant pattern. The LD₅₀ of the methanolic extract was calculated to be 245mg/kg/body weight an indication of mild toxicity.

In vivo anti trypanosomal effect showed that at 90mg/kg/body weight after clearing the parasite, the survival days of the animals also increased compared to other concentrations (40, 60, 70, 80mg/kg/body weight). The survival of the rats indicates a dose dependant pattern of the extracts, while the reason why the parasite got cleared up in the blood could be as a result of easy access of the extract to the parasites in the blood. The inability of the extract to penetrate the organs and to cross the blood brain barrier to kill the parasite there in must have led to a progressive breakdown of neurological functions which accomplishes the disease leading to the death of the animals as reported by Holland *et al* (2001).

Fraction 2 was more active than fraction 1, this could be as a result of the phyto compounds present in fraction 2 to be more active than those in fraction 1. Also in the *in vivo* treatment the parasitaemia was cleared from the blood on the 5th day post treatment using fraction 1 while for fraction 2 the parasitaemia was cleared on the 4th day post treatment there was not much difference in the effect of fraction 1 and 2 on the parasites *in vivo*. The survival time of the animals was also increased compared to the negative control (untreated). The antitrypanosomal properties of the extract are attributed to the presence of the phyto-compounds observed after GC-MS analysis mentioned in table 4.10 and 4.11. Since there was decrease in proliferation of parasitaemia and extension of surviving days of rats when treated with fraction 1 and fraction 2, compared to the negative control, it could be due to the presence of n-Hexadecanoic acid and Octadecanoic acid which has been reported to show significant antitrypanosomal properties as reported also by Oluwatosin, *et al* (2011). 1-Eicosene and 17-Pentatriacontene present in the fractions can also be responsible for the antitrypanosomal property of the fractions this is because they can be classified as simple aliphatic which have been previously reported by Perez *et al* (1994) to show significant suppression of trypanosoma parasitaemia *in vivo*.

The consistent parasitaemic suppression combined with prolonged time of survival of the experimental rats displayed by the *in vivo* test might be due to the ability of the extract to inhibit glycolysis, which is the major source of energy for the blood stream forms of trypanosome. However, the inability of the extract to produce curative effects might have resulted from inadequate tissue concentrations of the active components of the extracts or the distribution of the parasite in animals (Fairlamb, 1982). This may further explains why the

massive parasitaemia observed in the negative control group resulted into severe effects that threatened the survival of the rats.

The results of this current study have also indicated that the trypanocidal activity of the methanolic extract which was expressed in the *in vitro* compared to the *in vivo* study. This may be attributed to the possible deactivation of the trypanosomal properties of the extract as a result of *in vivo* metabolism following parental administration of the extract in the albino rats as has been reported with some trypanosomal drugs (Rang *et al.*, 1999).

In the prophylactic studies of the methanolic extract of *Hymenocardia acida* against *T. evansi* it was observed that the groups previously injected with the extract i.e. given 2 and 3 doses did not express parasitaemia even after 30 days. This may be due to the fact that the animals in the group after receiving the extracts for 3 days developed a resistance state for *T. evansi* even before they were challenged with the parasite. The prolonged administration of the extract for 3 days may have increased the immune response mechanism of the animals, which enable the immune system of the animals to combat and resist the expression of *T. evansi*.

The packed cell volume PCV of the rats treated with the extracts decreased significantly ($P < 0.05$) during the course of treatment except for the control being treated with Diminaveto[®] in which there was an increase in PCV. The decrease may be as a result of anaemia which is the most outstanding clinical and laboratory feature of African trypanosomosis (Suliman and Fieldman, 1989) and also the primary cause of death (Losos and Ikede, 1972; Mamo and Holmes, 1975). Anemia as indicated by PCV level is known to worsen with increasing parasitaemia (Ogbadoyi *et al.*, 1989). Though the *T. evansi* infected rats treated with 90mg/kg

of the extract was able to overcome the infection after 10 days of treatment but anaemia was still very much present and this may be the reason for death 16 days post treatment. Anaemia could also be the reason for the death of other animals during the course of treatment and after treatment. This shows that the methanolic extract of *Hymenocardia acida* was not able to improve the PCV even after clearing the parasites completely.

The actual mechanism by which the extract used was not investigated in this study, however trypanocidal activities from plant extracts have been shown to involve interference with trypanosome redox balance by acting either on the respiratory chain or on the cellular defenses against oxidative stress (Sepulveda-Boza and cassels, 1996). This effect is attributed to possession by natural product of structures that are able to generate radicals that might cause peroxidative damage to trypanothione reductase that is very sensitive to the alterations in redox balance. In addition trypanocidal agents have been known to bind kinetoplast DNA of the parasite (Hopp et al 1976; Sepulveda Bova and Cassels, 1996). It has also been reported that existing trypanocidal drugs exert their therapeutic action through a variety of mechanisms thus while arsenic compounds poison the cells by acting on glucose catabolism through glutathione, suramine targets glycolysis in the glycosomes, while pentamidine and other diamines disrupt the kinetoplast, which may also interfere with polyamine synthesis, other drugs like eflornithine are selective inhibitors of ornithine decarboxylase, depleting the biosynthesis of polyamines such as spermidine, a precursor of trypanthione (Nok *et al.*, 1993; Atawodi, 2004).

Partially isolated components from fraction 2 showed the combination of Benzenepropanoic acid,3,5-bis (1, 1- dimethylethyl)-4-Hydroxy octadecyl ester which had a concentration of 34.02% which is a complex antioxidant a type of molecule that neutralizes harmful compounds called *free radicals* that damage living cells (Muanza *et al* 1993). Fraction 1 showed the combination of 3',8,8',-trimethoxy-3-piperidyl-2-2'-binaphthalene-1',4,4;- tetrone which had a concentration of 25.53%, it is used as an analgesic and anti-inflammatory agent (Hoet *et al.*, 2004b). This confirms the use of *Hymenocardia acida* in ethnomedicinal practice in Nigeria. In the treatment of trypanosomosis, although the particular pure compound responsible for the antitrypanosomal activity was not purified, the trypanosomal properties may also be as a result of these compounds acting individually or synergistically to bring about the observed trypanosuppression activity in the fractions (Hoet, *et al* 2003). It is also possible that some compounds present could have antitrypanosomal properties but have not been discovered.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The major findings of this work can be summarised as follows;

- I. The methanolic extract of the stem bark of *Hymenocardia acida* have been shown to be toxic at 245mg/kg/body weight
- II. The Phytochemical screening of the methanolic extract of the stem bark of *Hymenocardia acida* revealed the presence of alkaloids, tannins, steroids, carbohydrates, flavonoids, glycoside`s, saponnins, tretipenes, and sugar.
- III. Anti-trypanosomal screening of the extracts showed that petroleum ether extract had no effect on the parasites while chloroform and methanolic extract had appreciable effects on the parasite, with methanolic extract being more active.
- IV. In the *in vivo* studies of the methanolic extract at 90mg/kg/body/weight was able to appreciably reduce the parasite load.
- V. The methanolic extract was partially purified using chromatographic techniques and GC-MS carried out, it revealed the presence of n-Hexadecanoic acid and Octadecanoic acid. Also, 1-Eicosene and 17-Pentatriacontene can be classified as simple aliphatic may be responsible for the antitypanosomal properties.

6.2 Conclusion

This study provided some insight on the acute toxicity and antitypanosomal activity of *H. acida*, the observed *in-vivo* anti-tyrpanosomal activity of the stem bark methanolic extract of this plant supports earlier reports that some plant extracts possess *invivo* activity against trypanosomes. This could also support the scientific basis for the traditional use of *H. acida* in the management of trypanosomosis.

In conclusion considering the effects of the plant extract in this study and the need for appropriate control measures against trypanosomosis the plant *Hymenocardia acida* can be said to have some anti-trypanosocidal activity on *T. evansi* and could be said to be a potential source of new drug in the treatment of trypanosomosis. However, available, information points to the fact that a number of tropical plants contain constituents that have been demonstrated to be clinically efficacious against many protozoa diseases. It is therefore clear that more work needs to be carried out in order to establish the usefulness of these plants in therapeutic preparations.

6.3 Recommendation

Further work is needed to be done in order to isolate and purify specific compounds responsible for the antitrypanosomal activity of *H. acida*

The methanolic extract of *Hymenocardia acida* should be tested on other trypanosomes such as *Trypanosoma congolense*, *Trypanosoma equiperdum* and *Trypanosoma vivax*.

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Appendix 3 In vitro antitrypanosomal properties of the chloroform extract of *Hymenocardia acida* on *Trypanosome evansi*

	Concentration of parasitaemia (antilog 1×10^6 parasites per ml) per minute												
Concentration of extract	1	2	3	4	5	6	7	8	9	10	11	12	13
100	6.9	0	0	0	0	0	0	0	0	0	0	0	0
80	8.4	8.1	7.5	0	0	0	0	0	0	0	0	0	0
40	8.4	8.1	8.1	7.8	7.8	7.5	6.9	6.9	0	0	0	0	0
20	8.7	8.4	8.1	8.1	7.8	7.2	7.2	6.3	6.0	0	0	0	0
10	8.7	8.4	8.4	8.1	8.1	7.8	7.2	7.2	6.3	6.3	6.0	6.0	0

Appendix 4 In vitro antitrypanosomal properties of the methanolic extract of *Hymenocardia acida* on *Trypanosome evansi*

Concentration of extract	Concentration of parasitaemia (antilog 1×10^6 parasites per ml) per minute			
	1	2	3	4
100	0	0	0	0
80	0	0	0	0
40	0	0	0	0
20	0	0	0	0
10	8.7	7.8	0	0

Appendix 5 In vitro antitrypanosomal properties of the petroleum ether extract of *Hymenocardia acida* on *Trypanosome evansi*

Concentration of extract	Concentration of parasitaemia (antilog 1×10^6 parasites per ml) per minute			
	1	2	3	4
100	8.7	8.7	8.7	8.7
80	8.7	8.7	8.7	8.7
40	8.7	8.7	8.7	8.7
20	8.7	8.7	8.7	8.7
10	8.7	8.7	8.7	8.7

Appendix 6 In vitro antitrypanosomal properties of fraction 1 on *Trypanosome evansi*

Concentration of extract	Concentration of parasitaemia (antilog 1×10^6 parasites per ml) per minute													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	8.7	8.4	7.8	0	0	0	0	0	0	0	0	0	0	0
20	8.7	8.4	8.1	8.1	7.8	7.2	6.9	0	0	0	0	0	0	0
10	8.7	8.7	8.4	8.4	8.1	8.1	7.8	7.8	7.5	7.5	6.9	0	0	0
5	8.7	8.7	8.4	8.4	8.1	8.1	7.8	7.8	7.5	7.5	6.9	6.9	6.3	0

Appendix 7 In vitro antitrypanosomal properties of fraction 2 on *Trypanosome evansi*

Concentration of extract	Concentration of parasitaemia (antilog 1×10^6 parasites per ml) per minute			
	1	2	3	4
100	0	0	0	0
80	0	0	0	0
40	0	0	0	0
20	0	0	0	0
10	0	0	0	0
5	8.7	7.8	0	0

Appendix 8 The R_f value of component 1 and 2

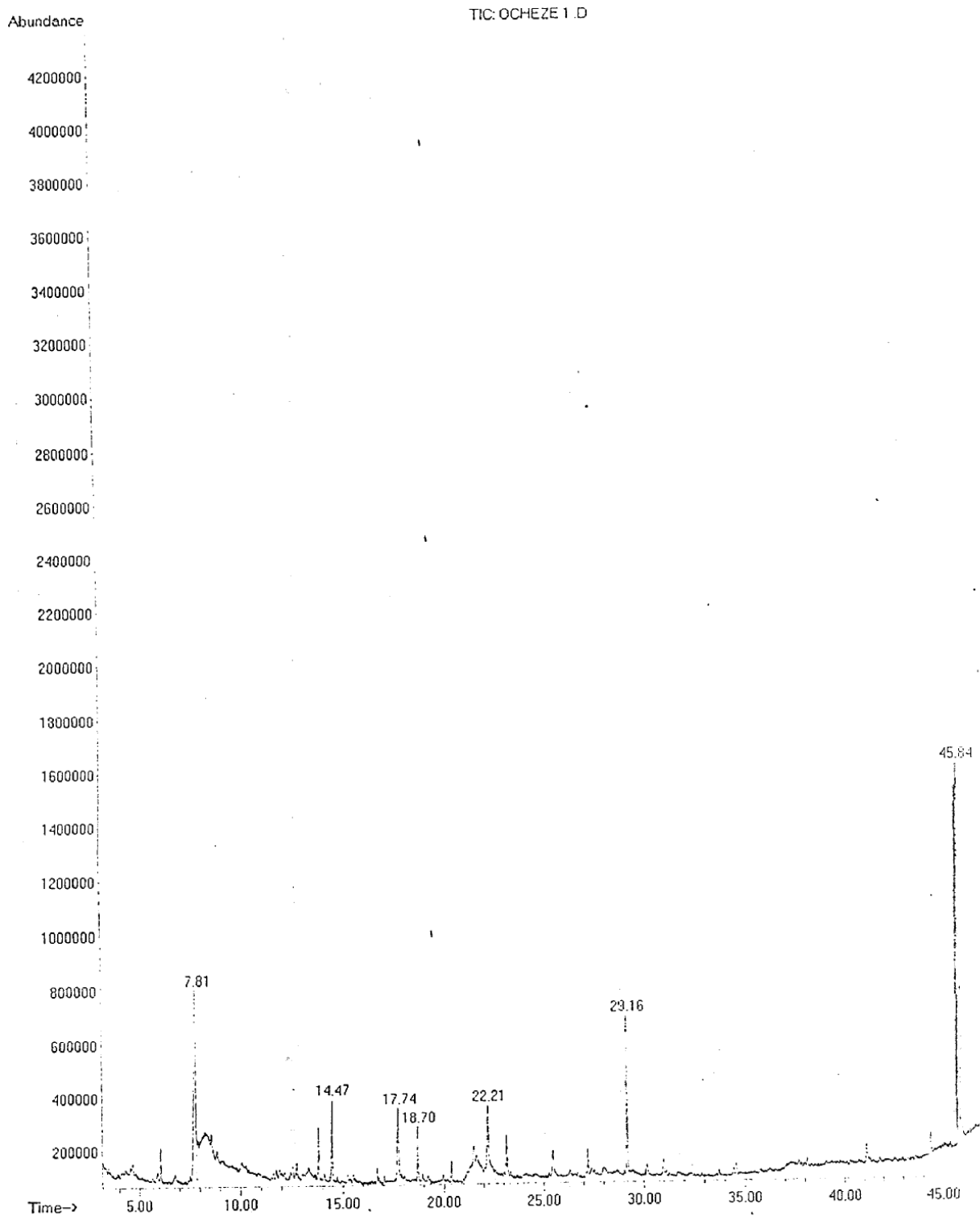
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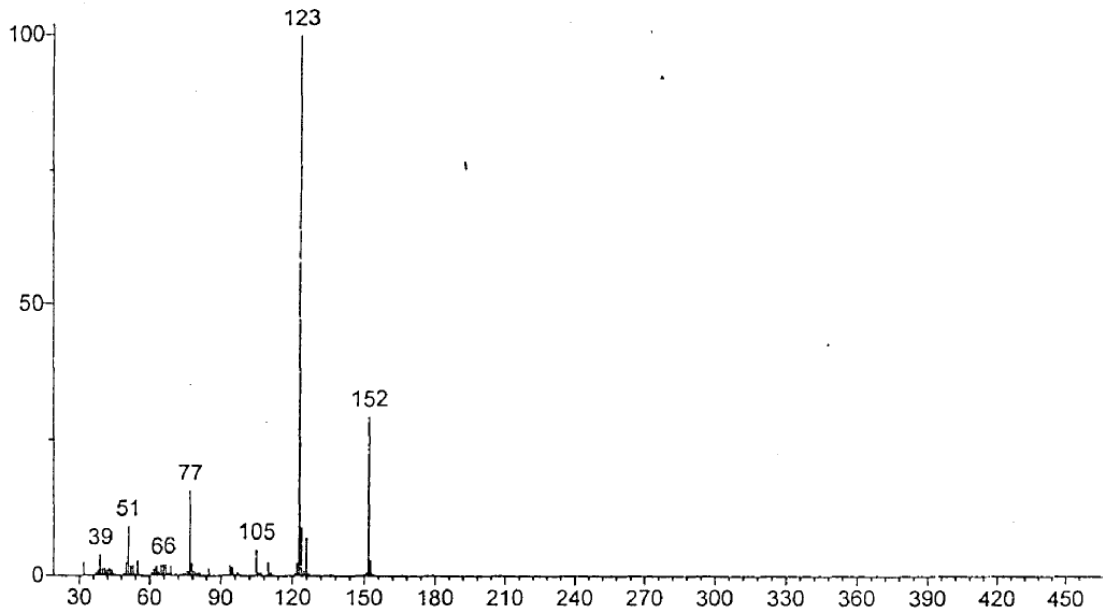
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Appendix 9 The R_f value of fraction 1 and 2

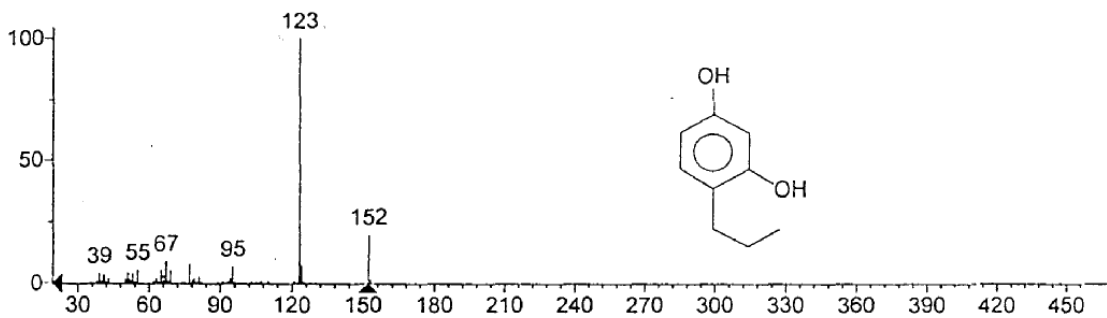
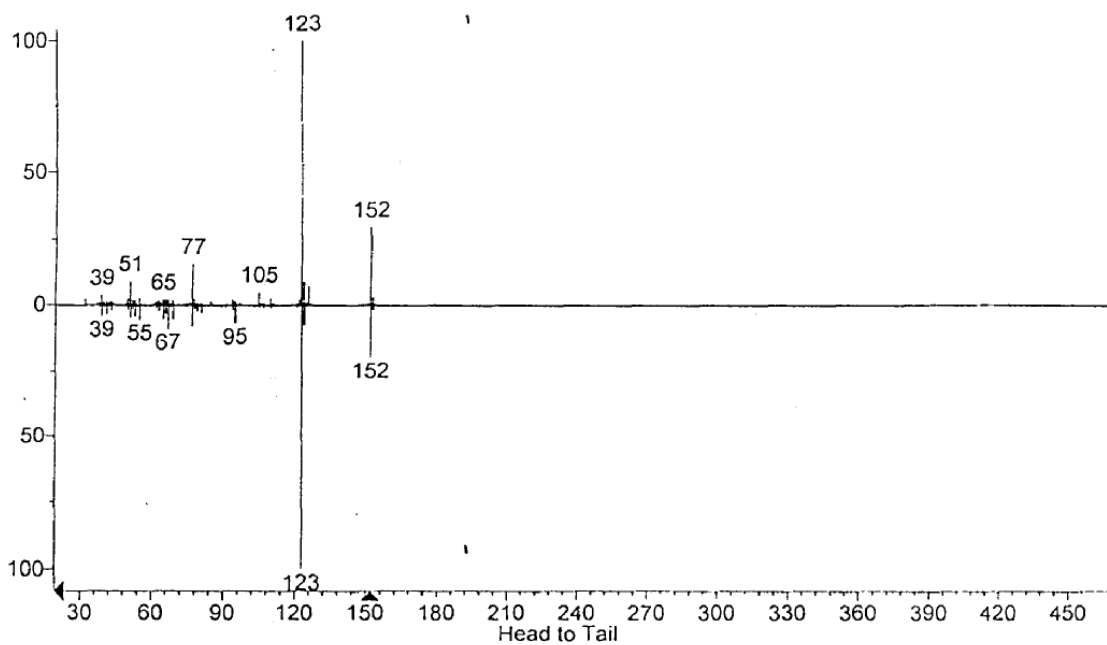
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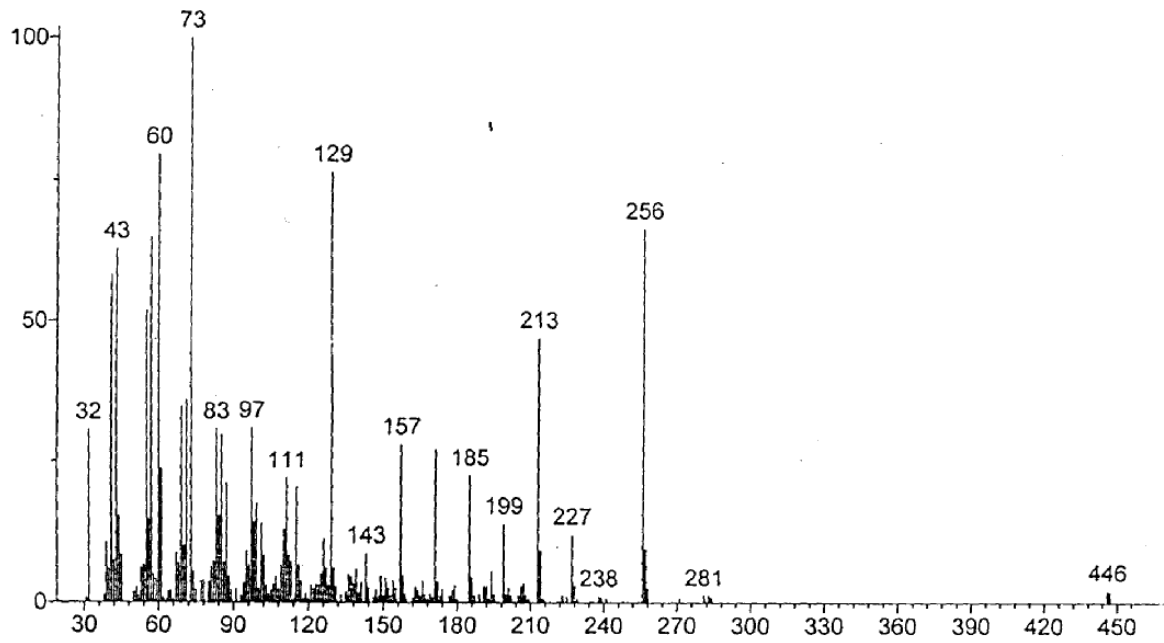




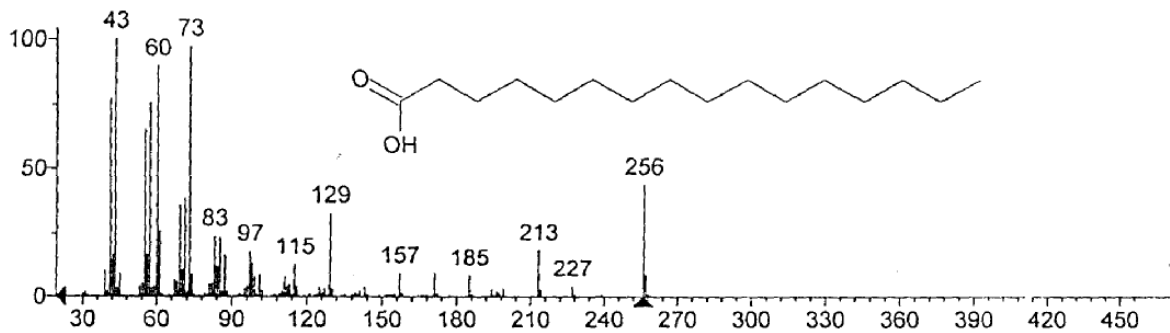
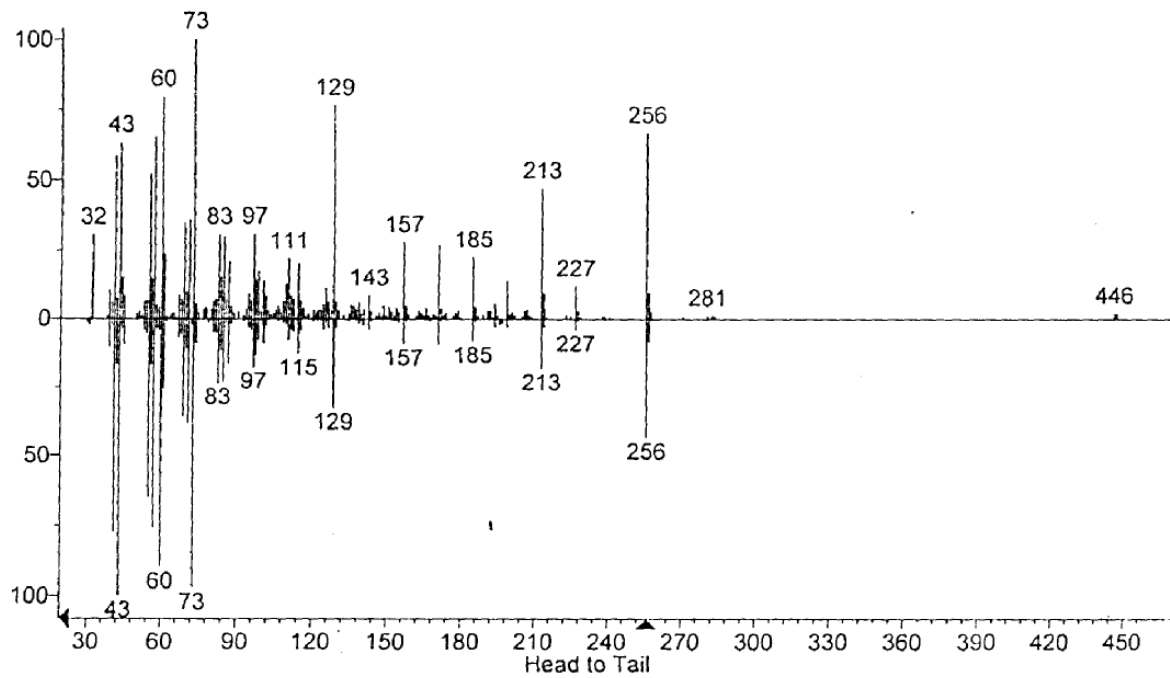
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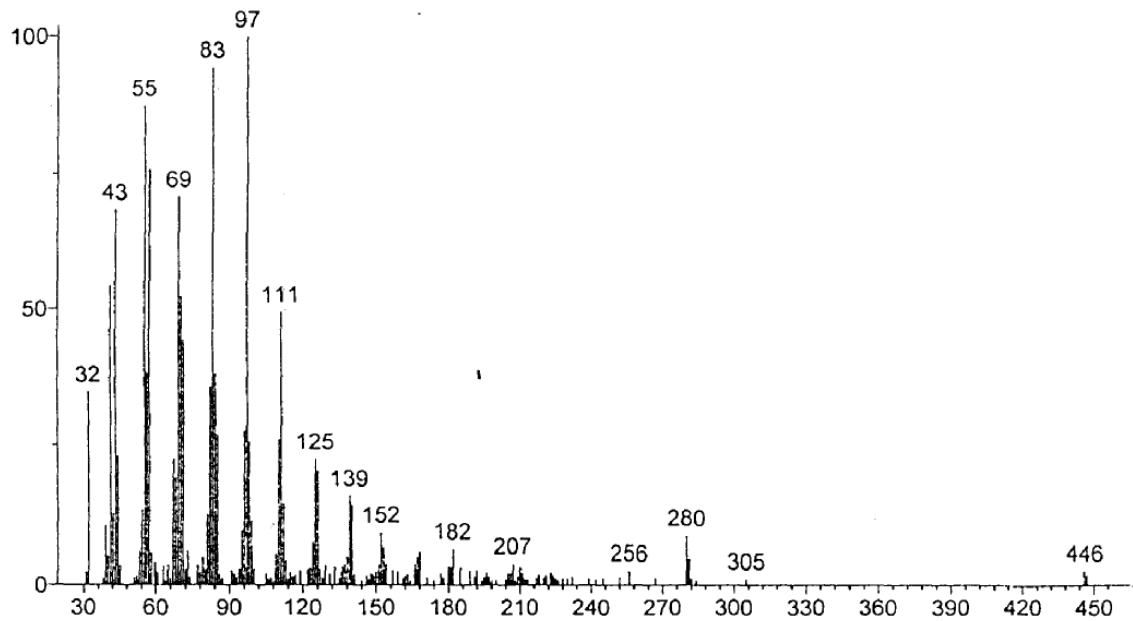
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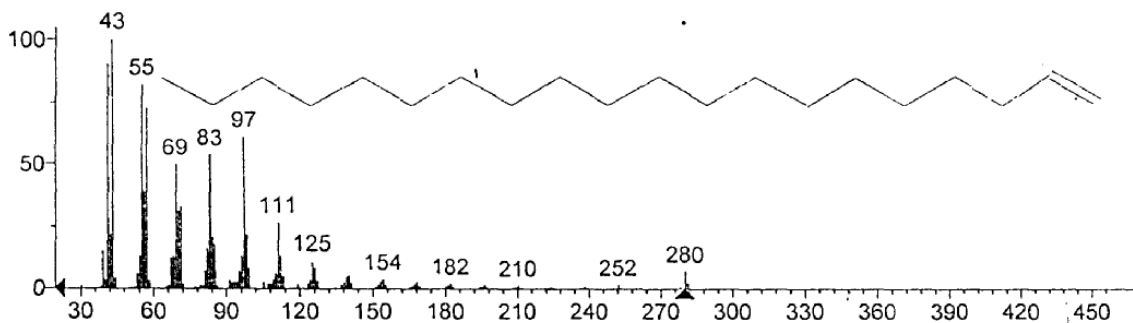
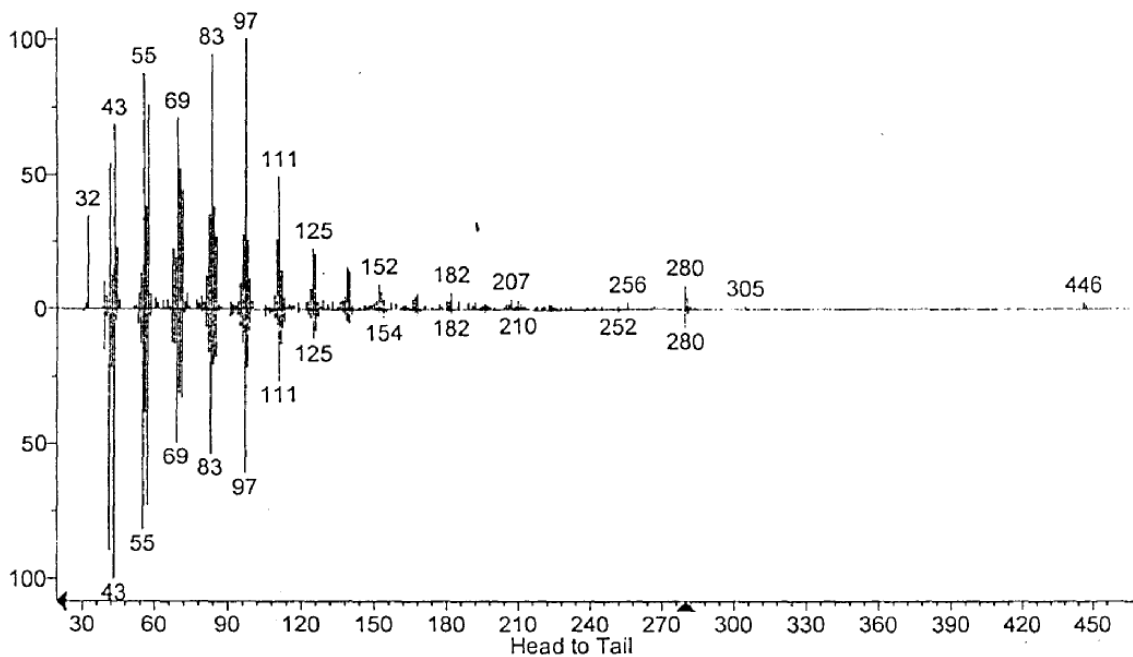
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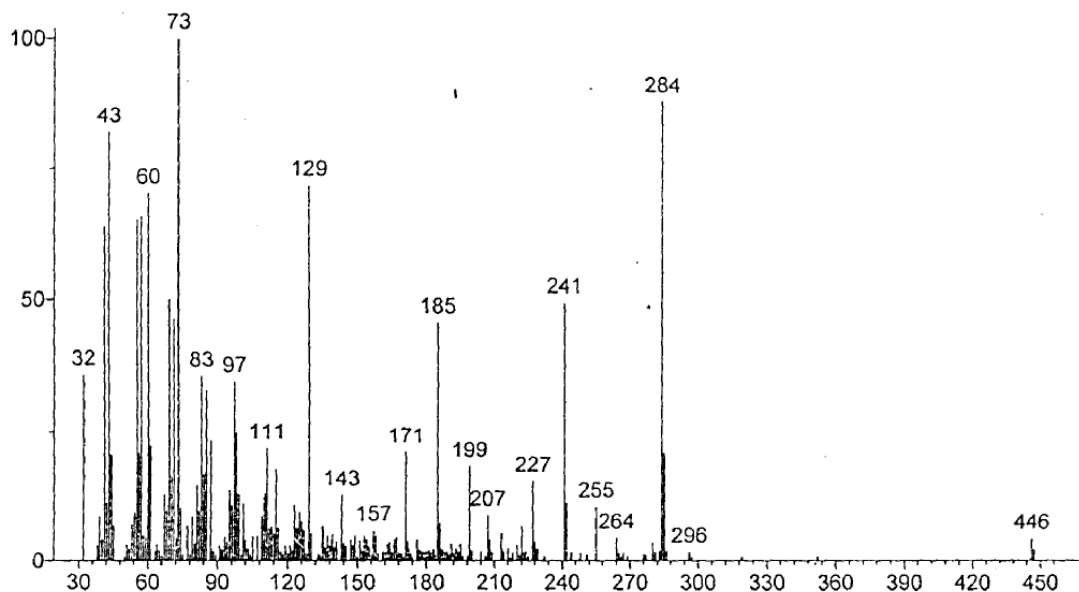
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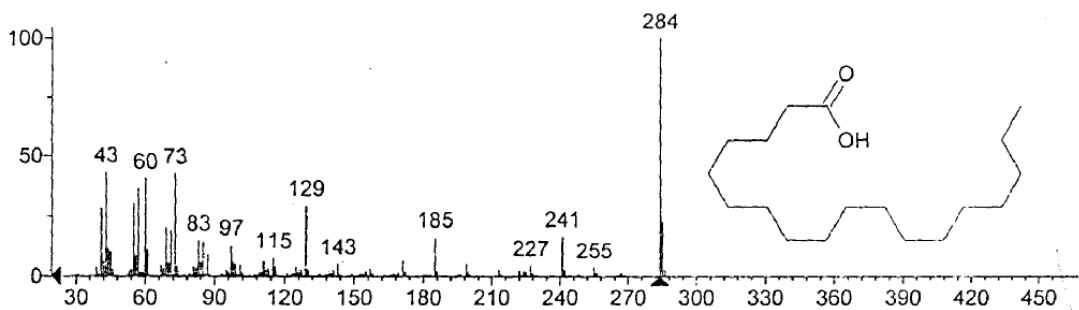
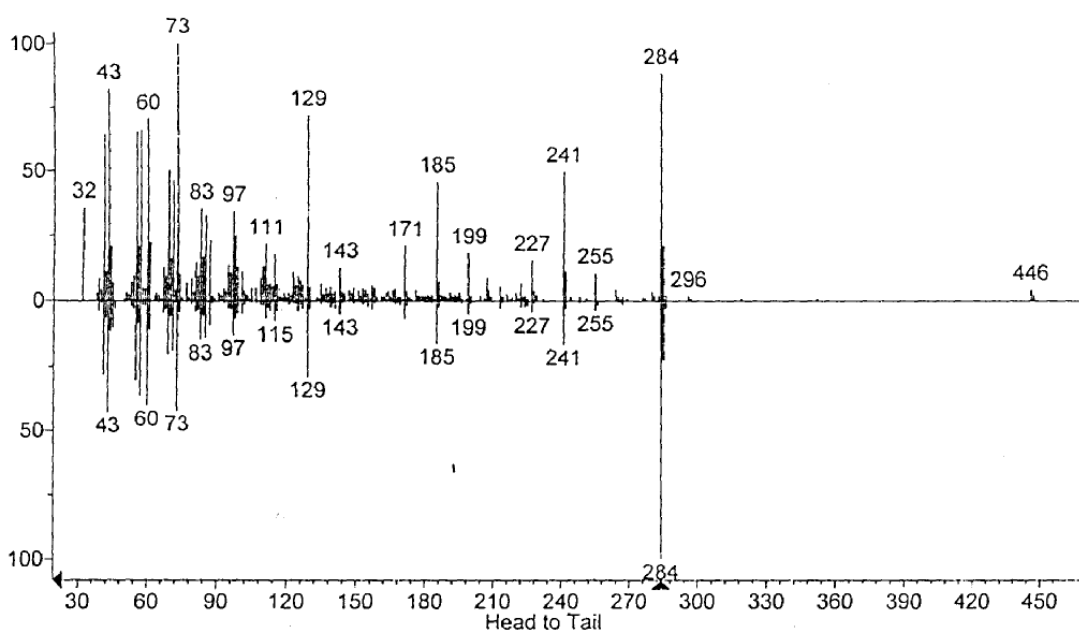
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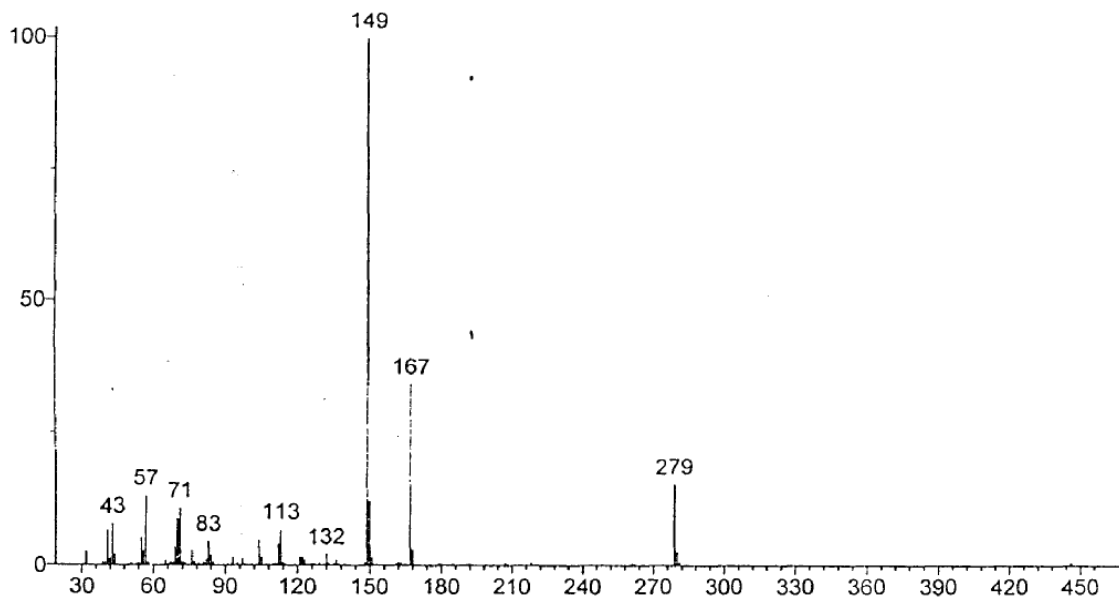
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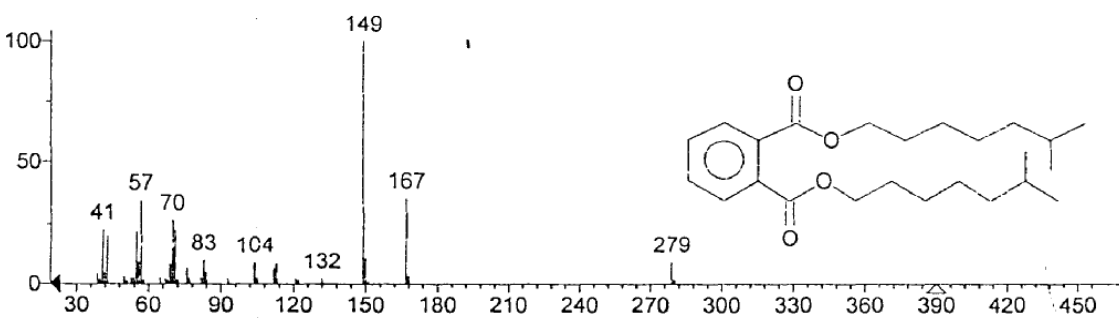
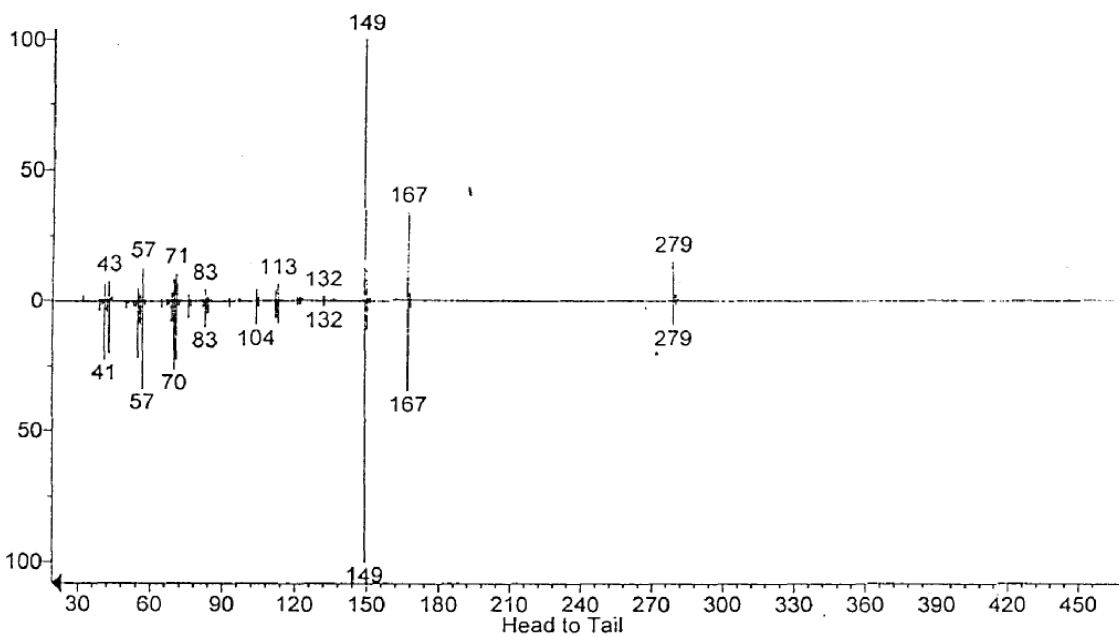
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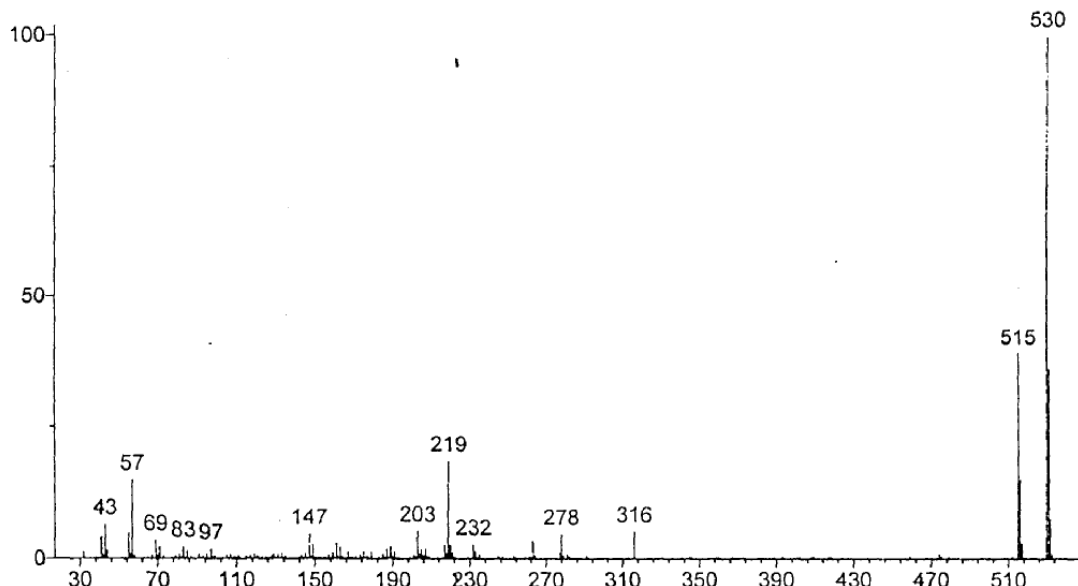
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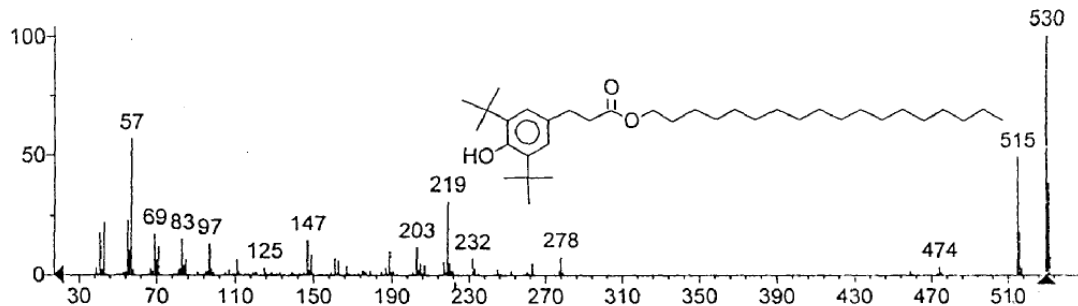
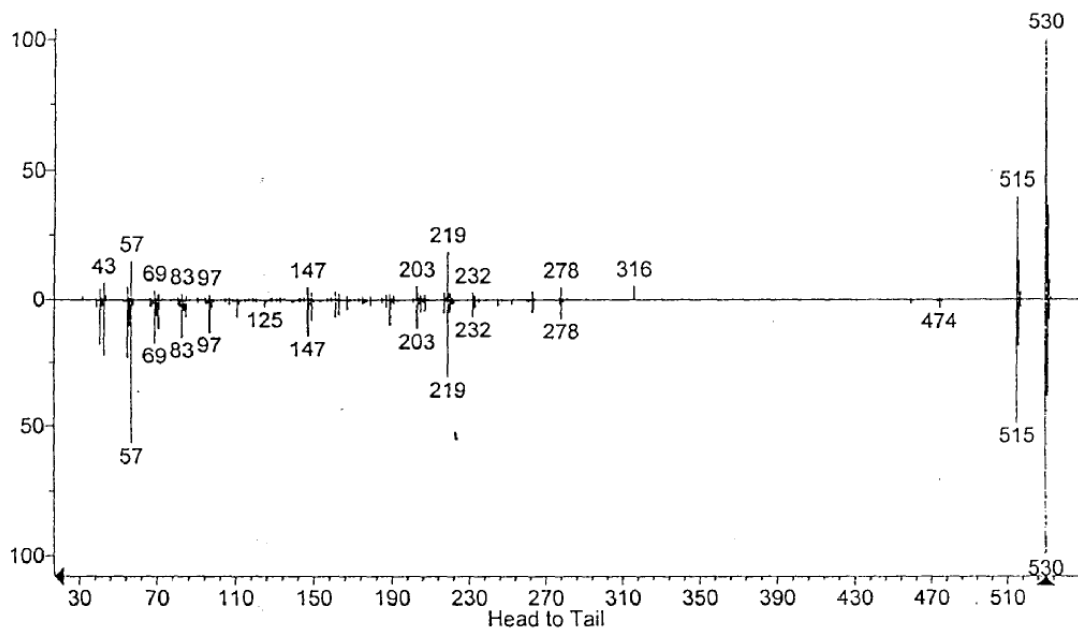
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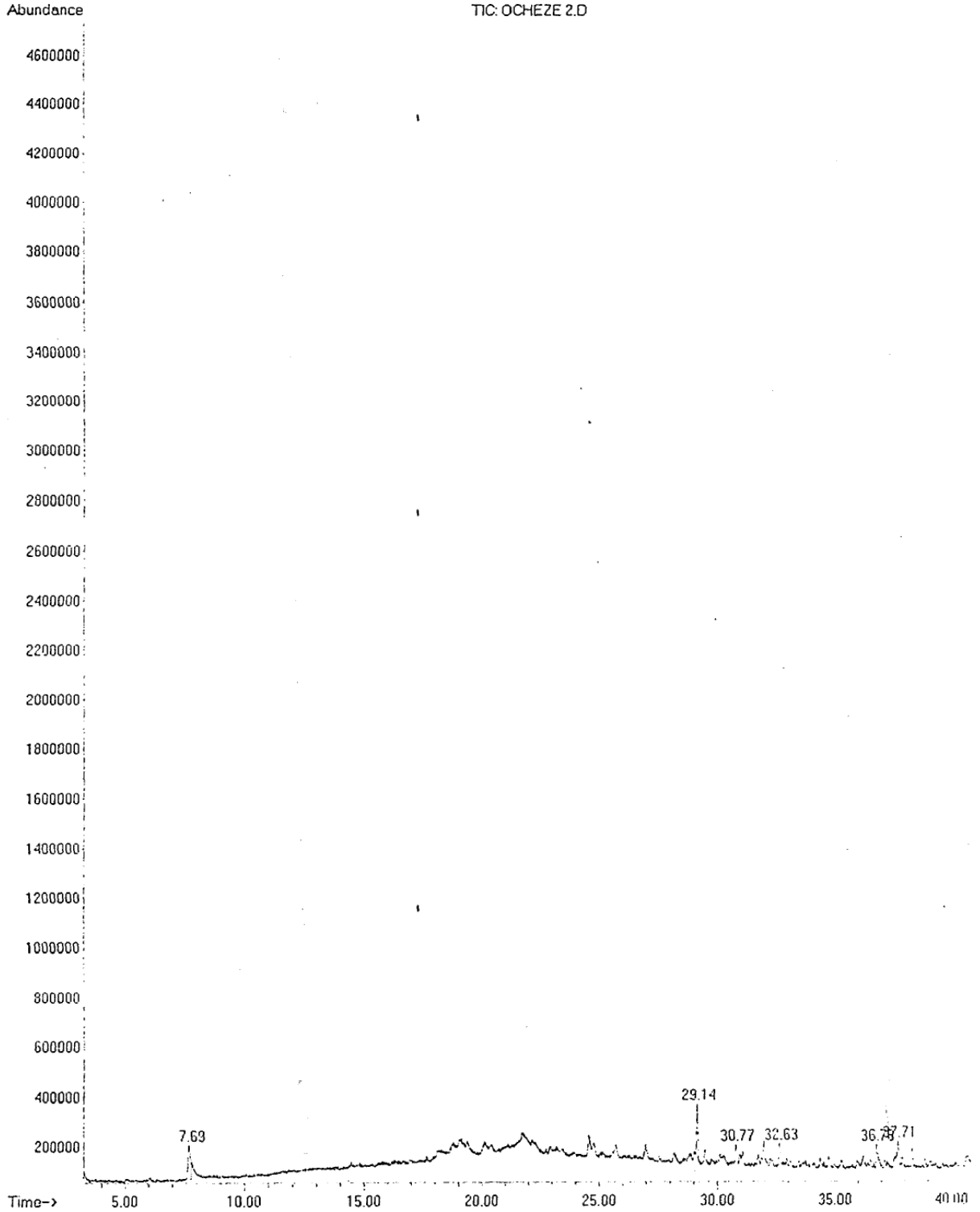


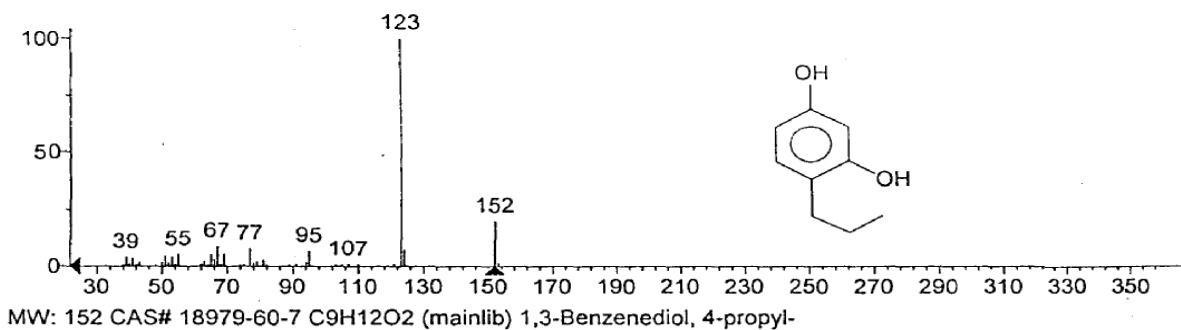
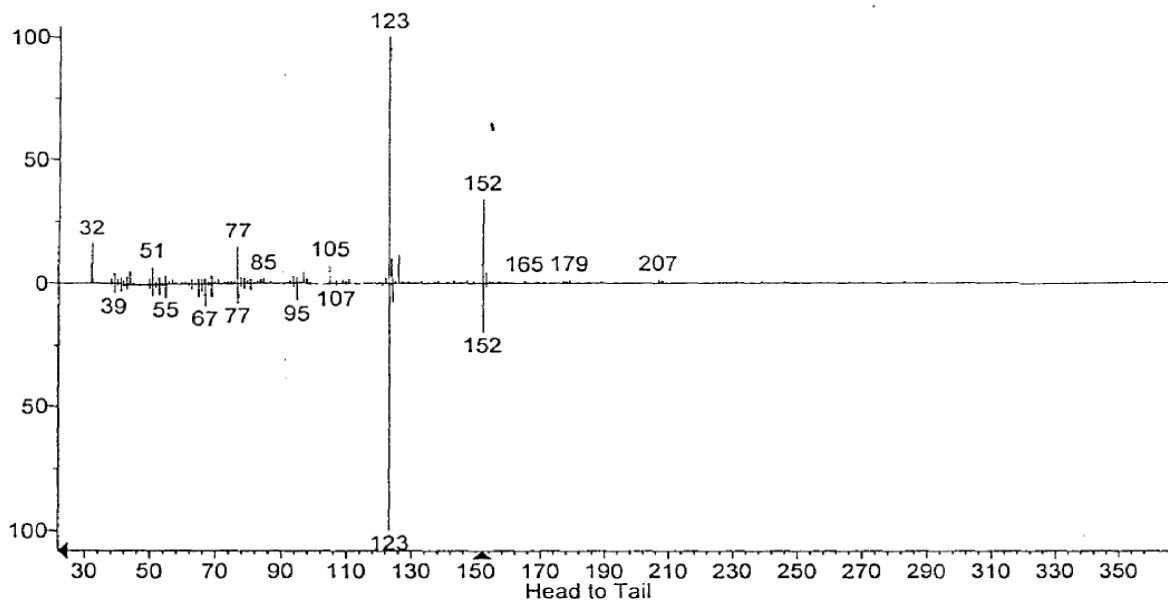
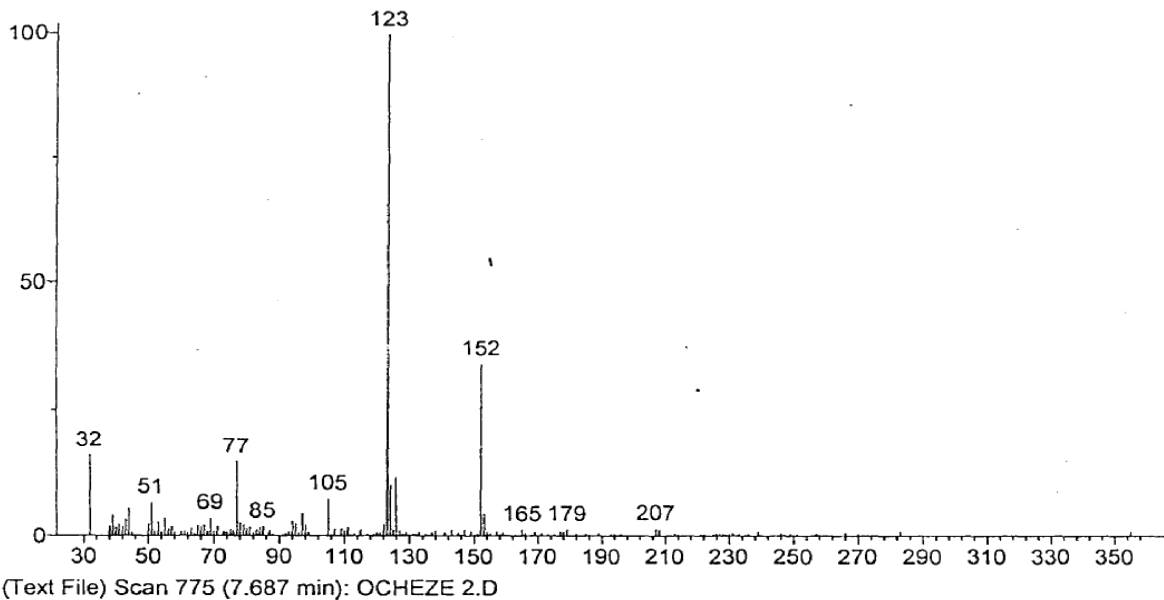
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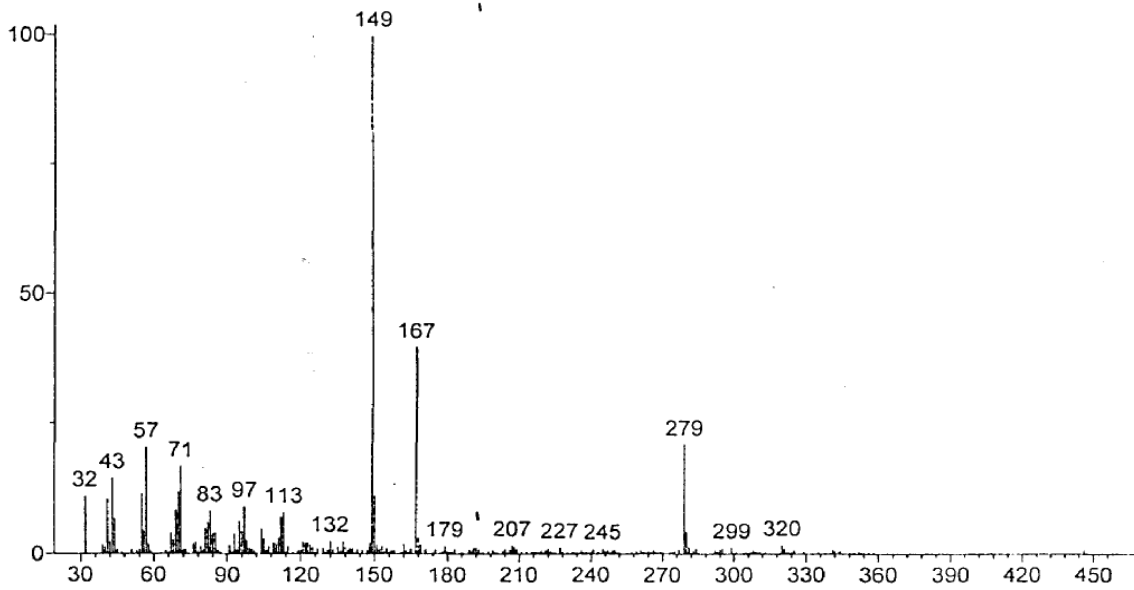


MW: 530 CAS# 2082-79-3 C35H62O3 (mainlib) Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester

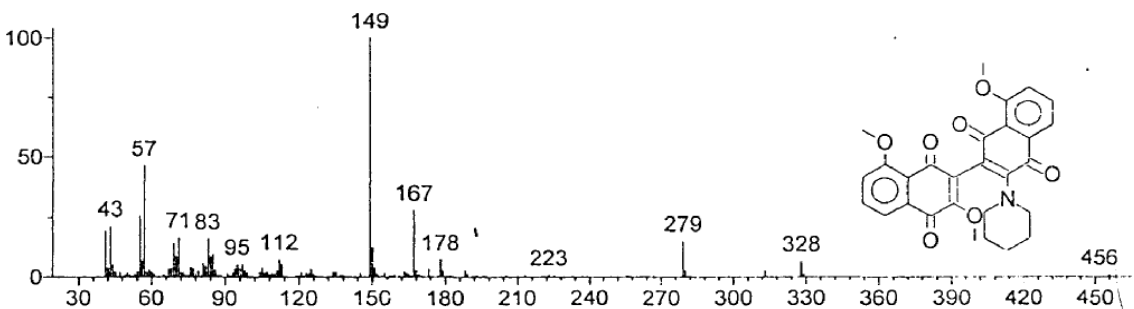
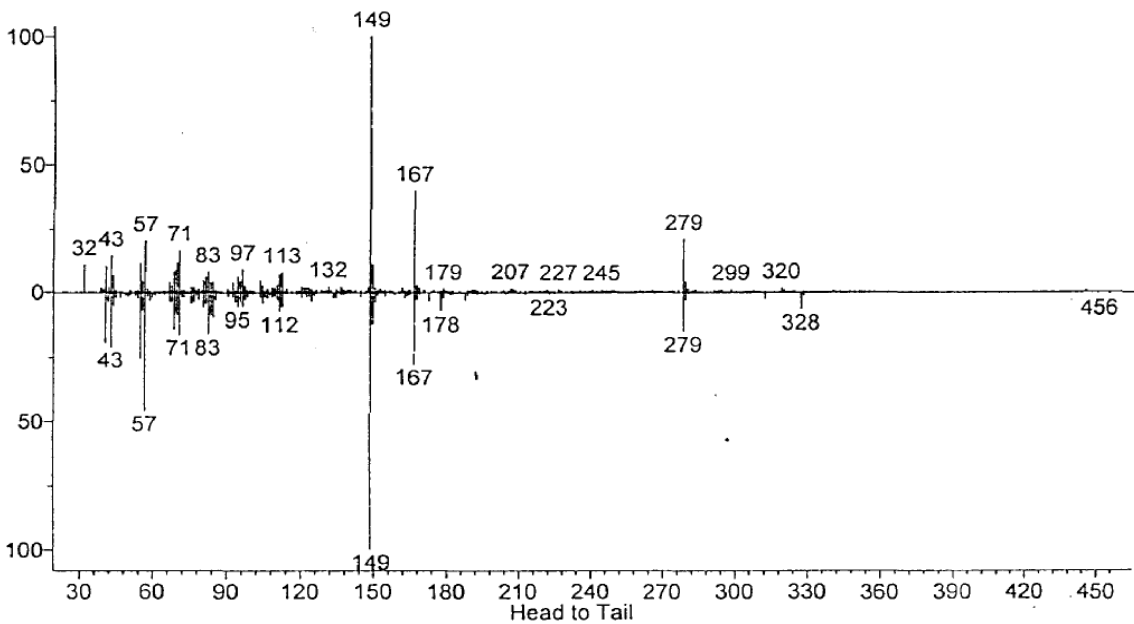
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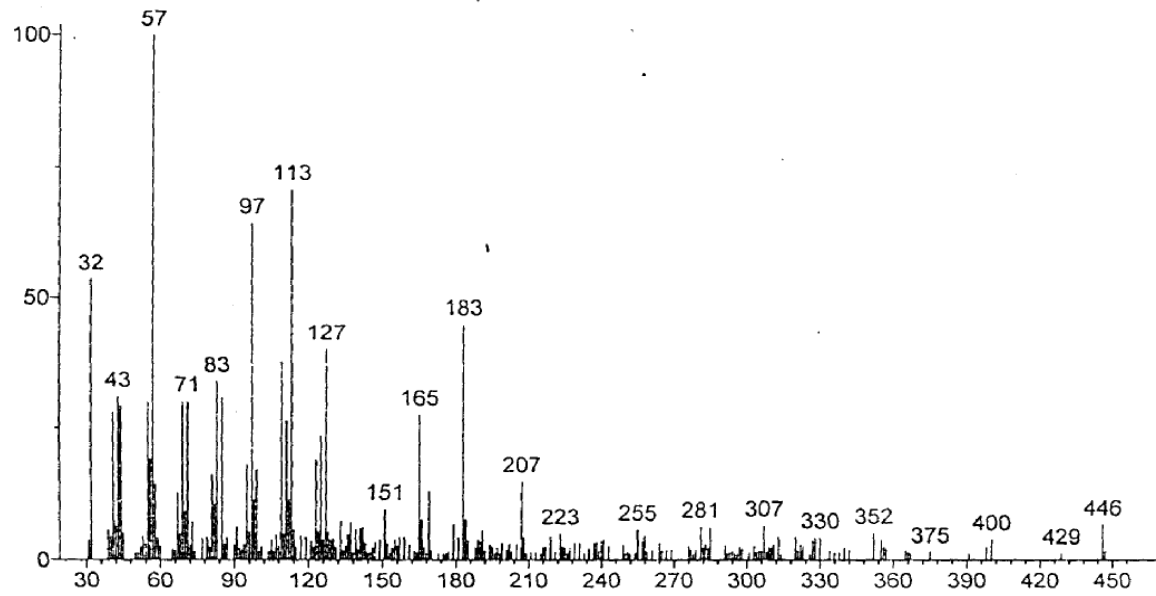




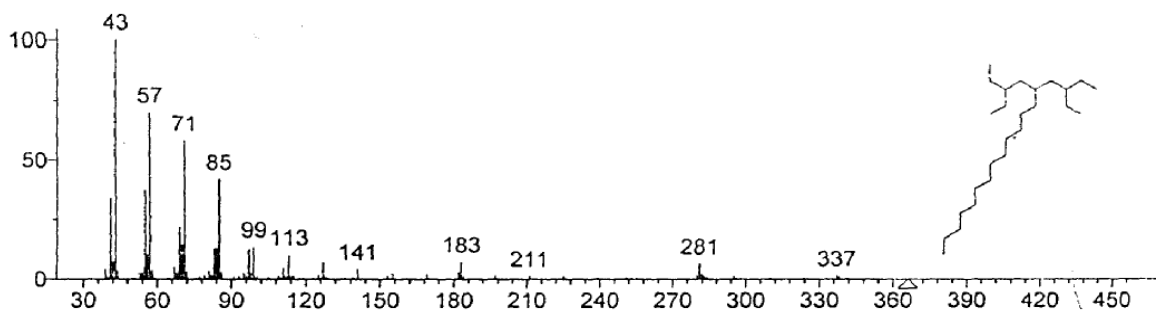
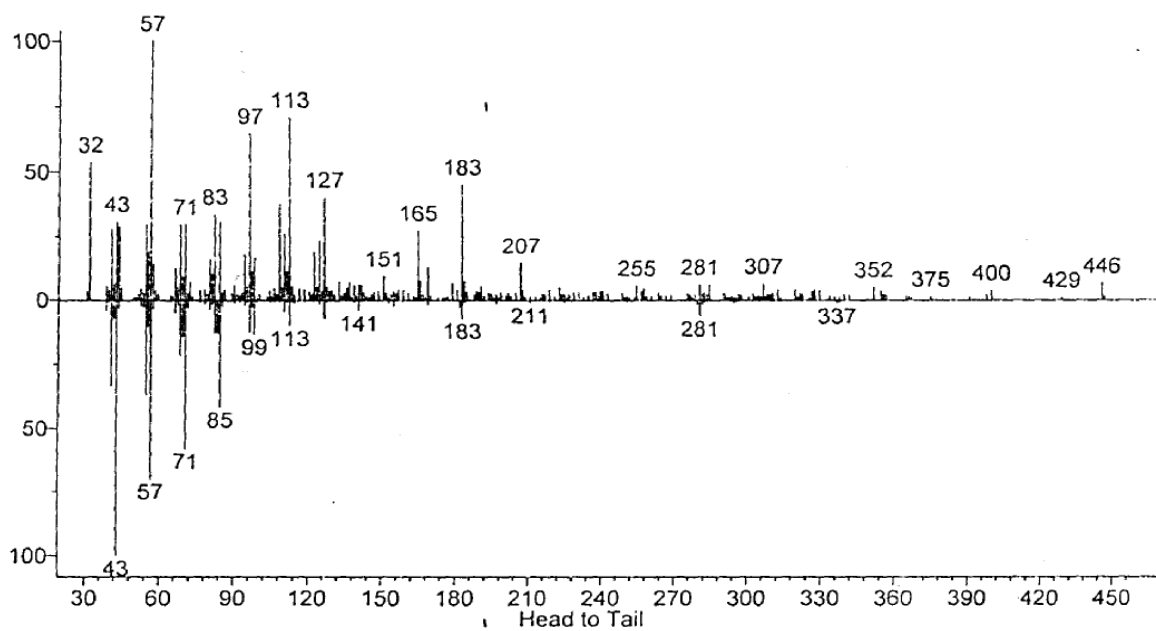
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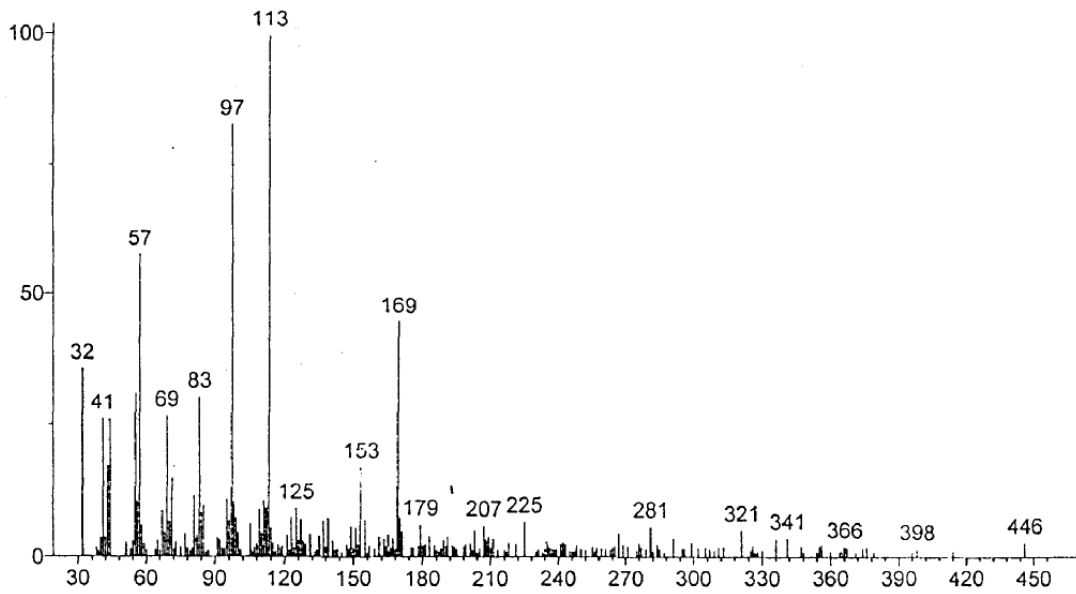
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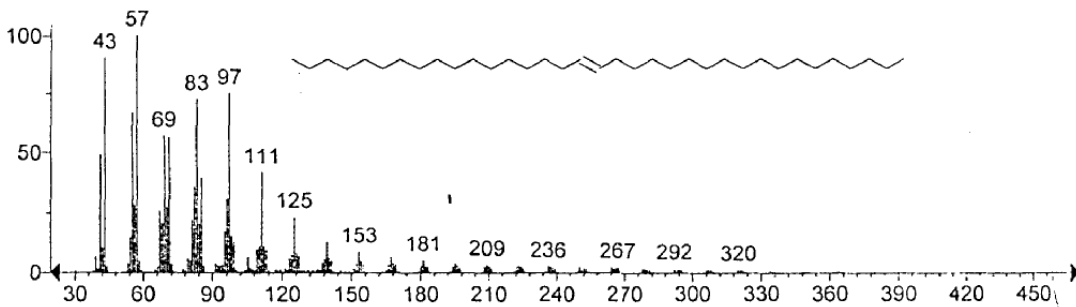
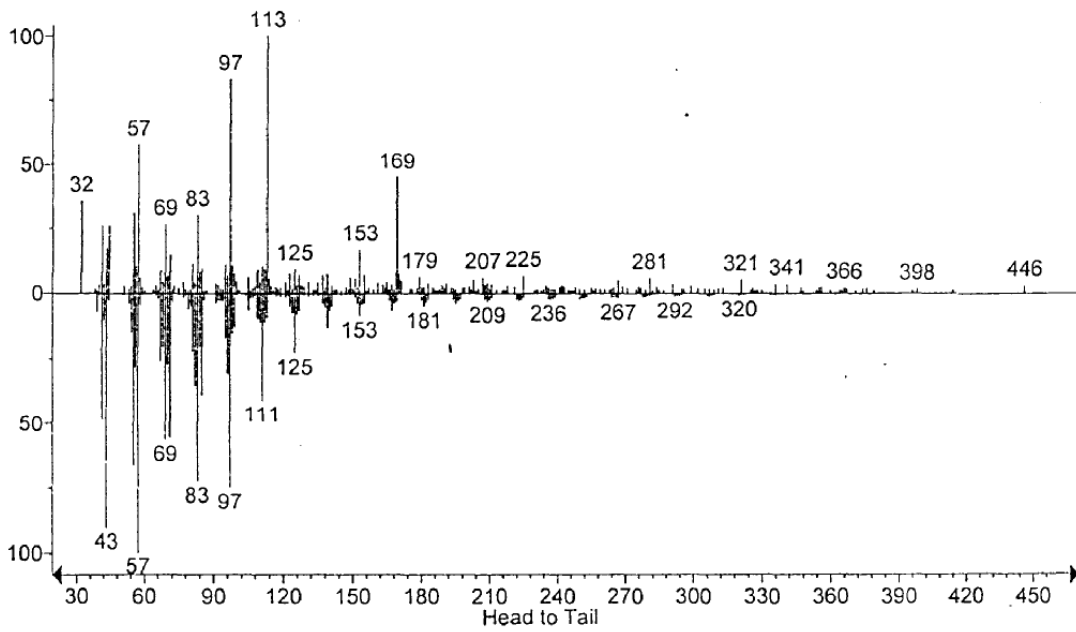
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MW: 366 CAS# 55282-12-7 C₂₆H₅₄ (replib) Octadecane, 3-ethyl-5-(2-ethylbutyl)-



(Text File) Scan 4979 (32.639 min): OCHEZE 2.D



MW: 490 CAS# 6971-40-0 C₃₅H₇₀ (mainlib) 17-Pentatriacontene

