THE ANTI-TERMITE PROPERTIES AND BASIC PHYTOCHEMICALS OF EIGHT LOCAL PLANTS AND THE CHEMICAL CHARACTERISATION OF *THEVETIA PERUVIANA* (PERS) K. SCHUM IN GHANA

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A thesis submitted to the Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, in fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

DECEMBER 2009

DECLARATION

I declare that this thesis is my own work, and that I have acknowledged all results and quotations from the published or unpublished work of other people.

Signed: Date: - 30 December 2009

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CERTIFICATION

This is to certify that this work is a true account of the candidate's own research.

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DEDICATION

Dedicated to my husband Harry and children - Elorm, Edem, Eyram and Elikem - for their love, patience and unflinching support. It is also dedicated to my mother Mary Ami Tordzro-Godzi for the support and encouragement to her children.

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ABSTRACT

There is an increasing interest in the use natural products for termite control because of their environmental safety. Some local plant materials have been mentioned as potential alternatives to synthetic termiticides. The objective of this work was to determine the antitermitic efficacy of locally available plants such as; *Thevetia peruviana* (pers) K Shum *Carapa procera* DC*, Jatropha curcus* L ,*Cassia nigricans* Vahl, C*ymbopogon ginganteus* (Hachst) Chiov), *Hyptis spicigera* Lam., V*etiver zizaniodes* Nash (vetiver grass) and *Chromolaena odorata* (L). Following the identification and collection of the experimental plants and termite samples, a series of field and laboratory experiments were conducted using parts of the plants to determine their antitermite efficacy. Antitermite efficacy was measured as their tolerance to termite damage, repellency and toxicity to termites. This was followed by extraction into petroleum ether, ethanol and water and the analysis of the most efficacious extract by chromatography (thin layer, column and high pressure) and spectrometry (mass spectrometry, nuclear magnetic resonance and infra red) methods to identify the active ingredients in the extract of the most efficacious plant. Resistance to termite destruction was measured by the loss in weight of stakes buried in treated and untreated soil and by visual assessment of extent of destruction. Repellency or attrantancy was determined by counting the number of termites that moved towards or away from filter paper pads treated with extracts of the test material. The results showed that soil treated with pulverised materials from *T. peruviana* offered the best protection to buried stakes against damage by subterranean termites. Field tests conducted with petroleum ether, ethanol and water extracts of *T. peruviana* suggested that the ethanol extract of *T. peruviana* resisted the destructive effects of termites most. In the repellency/attrantancy test, the ethanol extract was found to be an attractant. When the fractionated components of the ethanol extract were tested on brine shrimps, fraction 1 was found to be highly toxic suggesting obvious cytotoxicity. Analysis of fraction 1 by chromatography and spectrometry methods indicated the presence of two components digitoxin and digitoxigenin which were found to be toxic to brine shrimp. Sucrose was also isolated from the crude ethanolic extract of *T. peruviana.* Thus this work

has shown that the potential for the use of anti-termite agents from *T. peruviana* is promising.

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LIST OF ABBREVIATIONS

CC	**Column chromatography**
GC	**Gas chromatography**
HPLC	**High pressure liquid chromatography**
IR	**Infra red**
MS	**Mass spectrometry**
NMR	**Nuclear magnetic resonance**
TLC	**Thin layer chromatography**
UV	**Ultra violet**

CHAPTER ONE

1. INTRODUCTION

Termites are soft-bodied Arthropods described as social insects. They are usually classified at the taxonomic rank of order Isoptera. (Engel and Krishna 2004)

Termites are important because their activities impact positively or negatively on the environment and population. Termites contribute significantly to maintaining most of the world's ecosystems. They help break down and recycle wood and other plant materials producing organic matter while their tunnelling activities also help to aerate soils. Termite activities also results in patchy changes or improvement to soil composition and fertility. Compacted and encrusted soils will not support plant life but as termites tunnel through such soils, they help reclaim them. They also contribute significantly to atmospheric gases (Eggleton et al. 1999). Certain termite species in tropical countries grow fungus within their nests which develop into large mushrooms. These mushrooms which are totally cultured and cultivated by termites are eaten in some communities of Africa. Termites constitute a supplementary source of protein for man and most birds, lizards, frogs, and anteaters. Children and women also widely consume termite mound soil for nutritional or other benefits encouraged by indigenous belief systems (Sileshi et al. 2009).

These beneficial attributes notwithstanding, termites also impact negatively on the economy by causing damage to physical structures such as buildings, bridges, dams, railway sleepers, furniture, and even roads. They are also a threat to agriculture as they damage crops, forest trees and rangelands causing significant losses to annual and perennial crops. In buildings termites pose a great threat to structural timber and to the contents of buildings such as furniture, paper and clothing especially in the semi-arid and

sub humid tropics. In cases of severe infestations their activities result in the loss of structural strength of buildings. The damage caused by termites alone is reported to be more than the combined annual destruction caused by fires, tornadoes and earthquakes in monetary terms (Culliney and Grace 2000; Lewis et al. 2001; Lax and Osbrink 2003; Ahmed and French 2005; Isman 2006).

In Ghana, termites are found in the sub-soil almost everywhere and they cause considerable damage to vital infrastructure and property. However, reliable information on the economic losses caused by termites is difficult to obtain. A recent study conducted to investigate the extent of termite infestation in households in some communities in the country showed that there is a widespread incidence of termite infestation in buildings in Ghana and drastic control measures need to be taken to address the situation. In the Anwomaso community near Kumasi it was found that about 70% of the households have had some kind of termite infestation (Tagbor et al, unpublished).

Strategies of termite control vary greatly from place to place across the world. Generally, termite control is best achieved in buildings by providing physical and chemical barriers (Jones 2003; Su et al. 2004). There are various types of physical barriers and their implementation is based on the behaviour of target termites. The physical barrier method however does not exterminate the termites and if not properly constructed may be ineffective. The chemical control procedures include chemical treatment of the soil area, the application of preservatives for the preservation of wood and baiting. For example, the use of insecticides to treat soil to make it lethal or repulsive to termites and impregnation of timber prior to its use are effective against subterranean termites and dry

wood termites respectively. Various synthetic insecticides offer reasonable protection against termites (Spooner and Priest 1999; Smith et al. 2002). The use of these synthetic insecticides is however not without problems as demonstrated in Figure 1.1 below. Toxicity to non-target organisms, development of termite resistance to the substances used and health hazards due to resistance of these synthetic substances in the environment are some common problems associated with the use of synthetic insecticides (Kamble et al. 1992; Gamo et al. 1995; Chen et al. 2000).

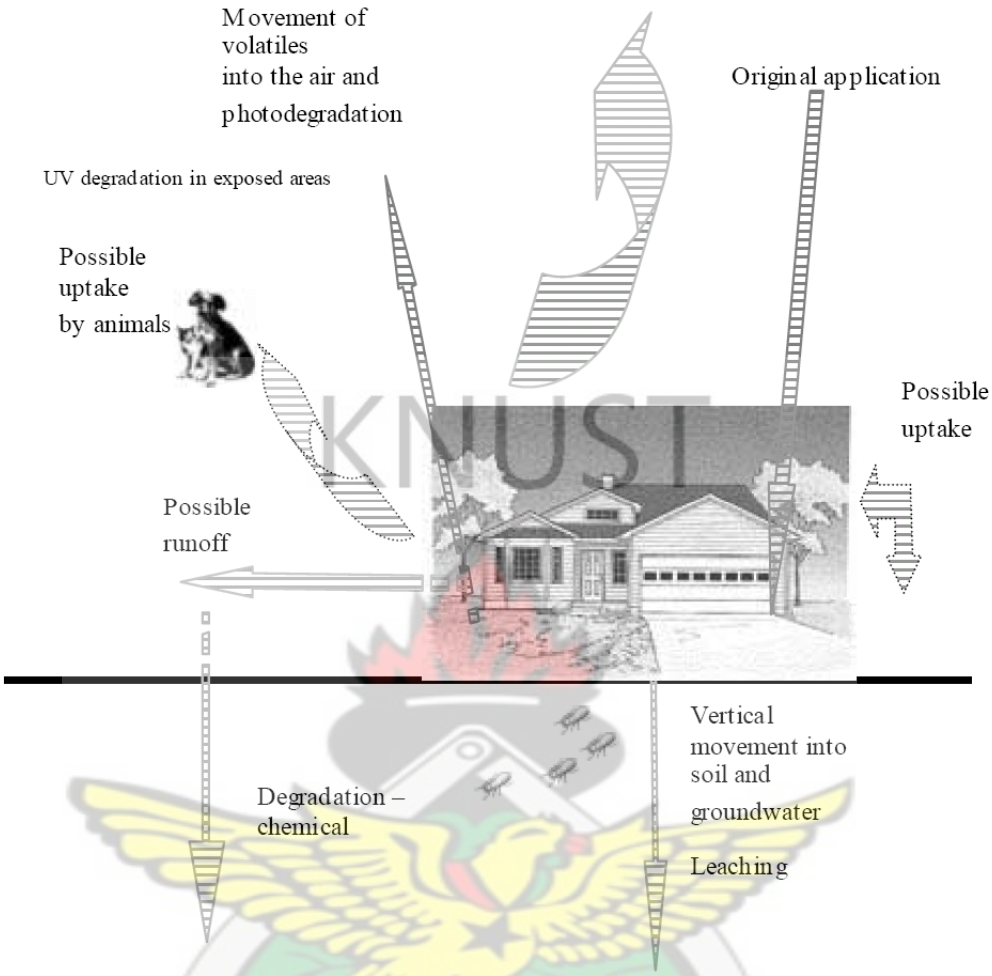


Figure 1-1: Generalised possible fate of termiticides after application to soil (http://www.free-clipart.net).

In Africa termite control using pesticides is likely to have negative impacts on human welfare and the environment. Direct exposure of farm families to pesticides could occur because people who apply pesticides usually do not take precautions or wear protective clothing. People who consume termites and mushrooms from treated termitaria could be exposed to pesticide residues and children and women can be exposed to pesticides through consumption of soil from treated termitaria. In addition, termite control practices could pose risks to non-target organisms that inhabit termitaria or consume the soil (Sileshi et al. 2009).

Problems associated with the use of pesticides have led to an increasing interest in the development of alternative termite control methods and plants with pesticidal properties may be one such alternative. Antitermitic activity has been observed in many hardwood (Angiosperm) species and plant extracts (Carter et al. 1983; Logan et al. 1990) and natural pesticides based on plant extracts have been commonly used in pest control during the earlier half of this century. Examples of some of these extracts are rotenone, nicotine and pyrethrum (Blaske and Hertel 2001; Maistrello et al. 2001; Ibrahim et al. 2004). Natural pesticides however lost their favour after the Second World War due to the introduction of more efficacious synthetic organic chemicals.

1.1 PROBLEM STATEMENT

Termites pose serious threats to agriculture, forestry and buildings in Ghana, where they are found in the sub-soil almost everywhere. The commonest method of controlling termite infestation in Ghana is the application of chemicals to the soil. Synthetically produced chemicals are the principal termiticides used for this purpose. However, there are problems associated with the large-scale use of these broad-spectrum synthetic pesticides, such as toxicity to non-target organisms, development of resistance to pesticides and environmental contamination which may affect the entire food chain (Fendick et al. 1990).

Lack of understanding of the proper use, adulteration, non-availability of suitable application equipment, inappropriate storage conditions and increasingly high prices of synthetic pesticides are additional problems that face those who use these synthetic chemicals in developing countries such as Ghana. There is an urgent need to investigate alternative termite controlling agents especially natural termiticides of plant origin

1.2 AIMS AND OBJECTIVES

The overall aim of this study was to evaluate eight locally occurring plants for their termite controlling properties and to identify the active component in the most efficacious one.

Specific objectives

1. To determine the natural termite controlling properties of eight local plants through 'Graveyard test' methods.
2. To determine the most efficacious plant material and it's most active extract through 'Graveyard test' methods.
3. To determine the basic phytochemical compositions of the plant materials and the extracts of the most efficacious plant.
4. To determine the mode of action (repellency/ attractancy/ toxicity) of the most efficacious extract to termite.
5. To identify the key chemical compounds responsible for the anti-termite properties of the most efficacious extract.
6. To determine the chemical structure of the major compounds responsible for the anti-termite properties of the extract.

1.3 STUDY JUSTIFICATION

Since termites are destructive and a constant threat to properties of individuals and governments, relentless efforts have been made to control their activities. Various control methods including application of termiticides, graded stones, glass splinters, stainless steel, chemical barriers and baiting systems are in use (Davis and Kamble 1992; Culliney and Grace 2000).

Although various parts of plants and materials obtained from them are reported to be either toxic or repellent to pests in general (Blaske and Hertel 2001; Maistrello et al. 2001; Peterson and Ems-Wilson 2003), no specific work has been done on the use of plant materials in the control of subterranean termites.

The most common method used in Ghana for the prevention and control of termite infestation, is the application of chemical termiticides to a structure's peripheral grounds (Ocloo 1998). The commonest chemical termiticides used for this purpose in Ghana are broad-spectrum synthetic pesticides including organophosphates and synthetic pyrethroids. Even though these synthetic pesticides play a significant role in controlling the destructive effects of termites, there are serious ecological and economic problems associated with their large-scale use. Related to this is the fact that termiticides are expensive and not locally available and need to be imported at high cost. (Kéïta et al. 2000).

It is worthwhile and prudent to begin scientific exploration for plant based natural products with anti-termitic properties. Botanical insecticides for example, can be used as alternative to synthetic insecticides. They are biodegradable, are often less toxic to

mammals and are less hazardous to the environment. Considerable attention has been given to natural insecticides of plant origin in recent years due to their effectiveness on many economically important insect species (Saxena 1989) and their environmental compatibility. Indeed research is now focused on these natural alternatives for pest control in developing countries (Kéita et al. 2001).

The selection of the most effective plant and its extract through bioassay and further identification of active component(s) proposed in this study will form a basis for termiticidal plants to be used in termite control. Findings obtained in this study could be used to embark on pilot production trials and testing with the ultimate aim of producing local alternative materials for the control of termites in Agriculture, forestry and real estate. The findings could also be used in formulating policies regarding the use of materials from plants as termiticidal agents.

1.4 SCOPE OF STUDY

Several activities and experiments were carried out to achieve the project objectives. These included firstly activities and experiments conducted to confirm the antitermitic properties of selected plants and secondly to characterize the chemical components of the most efficacious plant material.

Determination of antitermitic properties

1. Preparation of the different parts of the eight plant species
2. Collection and culturing of termite species in the laboratory
3. Bioassay of the ground plant parts against subterranean termite, *Macrotermis belliscos* (Smearthman).
4. Phytochemical screening of the different ground parts of the eight plants
5. Solvent extraction of plant material using three solvents of increasing polarity i.e. petroleum ether, ethanol and water
6. Determination of the most efficacious extract through bioassay
7. Phytochemical screening of most efficacious extracts

Characterization of the active component of most efficacious plant

1. Fractionation of the most efficacious crude extract
2. Determination of the most efficacious fraction through bioassay
3. Isolation of active constituents through chromatography methods

4. Identification of active constituents by Ultra Violet, Infra Red, Nuclear Magnetic Resonance and Gas Chromatography-Mass Spectrometry methods.
5. Determination of chemical structure and formulae of active constituents of efficacious extract

CHAPTER TWO

2. LITERATURE REVIEW

The chapter reviews literature on the followings termites; phytochemicals, chemical characteristics of antitermite compounds, extraction, separation and instrumental methods of analysis of plant derived products and plants with antitermitic activities.

2.1 TERMITES

In this section literature on the following were reviewed: termites, their biology and social behaviour, taxonomy and the various control methods.

2.1.1 BIOLOGY AND SOCIAL BEHAVIOUR

Termites (Isoptera) are eusocial insects characterized by (1) an overlapping of generations, (2) cooperative care of younger generations by older generations, and (3) presence of a reproductive division of labour, or a caste system (Lefebvre et al. 2008). Their highly successful eusocial nature is also evidenced by their historical success of over 200 million years and widespread distribution throughout many areas of the world (Hughes et al. 2008). Termites are soft bodied, pale in colour, with mouth parts for biting and chewing and utilizing cellulose as food source. They live in large colonies and depend entirely on wood, either living or dead, or the woody tissue of plants, intact or partially decayed (UNEP 2000).

A colony consists of reproductive forms, sterile workers, soldiers, and immature individuals. The reproductives are of two types, primary and supplementary. The primary reproductives, the king and queen, are pigmented and fully developed winged adults. Their role is egg production and dispersal by colonizing flights. The queen lives up to 25

years and lays about 3000 eggs a day through its enlarged abdomen (Thompson et al. 2000; Thompson et al. 2004; Husseneder and Simms 2008). The eggs are yellowish-white and hatch after 50–60 days of incubation. The colony reaches its maximum size in approximately 4–5 years and it may include 60,000 to 200,000 workers. In most termite colonies there is only one pair of primary reproductives, but when they die they are usually replaced by numerous supplementary reproductives, which are with or without wing pads and are slightly larger and more pigmented than workers. The sterile castes, the workers and the soldiers, are wingless and usually lack eyes (Husseneder et al. 2005). Worker and soldier termites are 6 mm long and pale cream in colour; however, the heads of soldiers are much enlarged (almost half their body length) with noticeable black jaws (Horiuchi et al. 2002). Workers construct the distinctive shelter tubes and collect food to feed the young and other members of the colony. Soldier termites are responsible for guarding the colony and its occupants (Higashi et al. 1991; Boomsma et al. 2005).

Termites continually groom each other to obtain certain secretions. These secretions help in regulating the number of individuals in the various castes (Philip 2004). Workers mature in a year and live up to 3–5 years. Soldiers also mature within a year and live up to 5 years (Myles 2005). Winged reproductives (alates) emerge in a mass nuptial flight in April and May. These flights are often the first indication of termite infestations (Philip 2004). After a brief flight, alates shed their wings. Females immediately search for nesting sites with males following closely behind. When the pair finds a moist crevice with wooden material, they form the royal chamber and lay eggs (Su and Scheffrahn 2000).

The work performed by individual colony members such as foraging, mound building, defence or reproduction is toward the success of the colony. All worker termites look for cellulose to feed on and forage in any material including plants, timbers and paper products. Thus workers find and bring food to the colony and feed all other colony mates (Calderon and Constantino 2007). Therefore, worker termites are the caste that causes all the damages on crops, buildings and structures (Vasconcellos et al. 2007). So worker termites are the important target for termite control (Higashi et al. 1991).

2.1.2 TAXONOMY

Termites have traditionally been classified separately by entomologists in an order called *Isoptera* (Engel and Krishna 2004; Husseneder et al. 2005; Calderon and Constantino 2007; Husseneder et al. 2008; Husseneder and Simms 2008). About 2800 termite species are recognized and classified in seven families (Aanen et al. 2002). These are arranged in a phylogenetic sequence; the first three families are the lower or primitive termites and the last four are the higher or advanced termites (Table 2-1). The Termitidae is the largest family of termites found worldwide and in Ghana. It includes mound-building termites and subterranean termites.

Table 2-1:An outline of termite classification (Engel and Krishna 2004)

FAMILY	SUBFAMILY	GENERA
Mastotermitidae		Mastotermes darwiniensis
Kalotermitidae		Kalotermes
Hodotermitidae	Carinatermitinaea	Carinatermes
	Lutetiatermitinae a	Lutetiatermes
	Hodotermitinae	Hodotermes
Termopsidae	Cretatermitinae	Cretatermes
	Porotermitinae	Porotermes
	Stolotermitinae	Stolotermes
	Termopsinae	Termopsis
Rhinotermitidae	Archeorhinotermitinaea	Archeorhinotermes
	Coptotermitinae	Coptotermes
	Heterotermitinae	Heterotermes
	Prorhinoterminae	Prorhinotermes
	Stylotermitinae	Stylotermes
	Termitogetoninae	Termitogeton
	Rhinotermitinae	Rhinotermes
Serritermitidae		Serritermes serrifer
Termitidae	Apicotermitinae	Apicotermes
	Foraminitermitinae	Foraminitermes
	Sphaerotermitinae	Sphaerotermes
	Macrotermitinae	Macrotermes
	Nasutitermitinae	Nasutitermes
	Termitinae	Termes

Termites inhabit approximately 70% of the world, mainly in the tropical and sub-tropical regions extending to some areas in the temperate region (Lee and Ryu 2003). There are now over 2700 species of termites described from 282 genera but these can be grouped in four major categories according to their nesting habitats and association with moisture. These are damp wood, dry wood, subterranean and arboreal termites (Haverty et al. 2005).

Damp wood termites also called wet wood termites live and feed on very moist wood especially stumps and fallen trees on the forest floor. They live in and feed on rotten logs

or highly moist timber in soil. Species in this ecological group are composed of two families of termites, Termopsidae and Kalotermitidae. The pest status for this group is minor compared to the other termite groups (Goulding et al. 1973).

Dry wood termites (Family: Kalotermitidae) are found commonly on most continents. They do not require contact with moisture or soil in order to survive. They nest entirely in timber above ground. Dry wood termite species vary in their ecology and biology. They infest dry, sound wood, including structural lumber, as well as dead limbs of native trees, shade and orchard trees, utility poles, posts, and lumber in storage. Dry wood termites have a low moisture requirement and can tolerate dry conditions for prolonged periods. They do not connect their nests to the soil. Piles of their faecal pellets, which are distinctive in appearance, may be a clue to their presence. (Bach 1990).

Arboreal termites also called mound builders are capable of building earthen towers eight meters or more in height above the ground. Their presence is indicated by mounds found commonly in Africa, Australia, Southeast Asia and parts of South America. The size of a mound also indicates their population size (Diehl et al. 2005).

Subterranean termites (Families: Rhinotermitedae and Mastotermitidae) live and breed in soil at varying depths. However, some subterranean termites may construct nests in trees or other above ground locations. They are found practically throughout the tropical and temperate parts of the world (Parman and Vargo 2008). Subterranean termites require a source of moisture in their environment. To satisfy this need, they usually nest in or near the soil and tend to reach their food sources from the underlying soil. They maintain some connection with the soil through tunnels in wood or through shelter tubes that they

construct. These shelter tubes are made of soil with bits of wood and termite faecal material (Haverty et al. 2005).

There are several genera of subterranean termites found in the literature. They include *Coptotermes Odontotermes, Microtermes, Recticulitermes and Hetrotermes* (Su et al. 2000; Haverty et al. 2005). Subterranean termites include the eastern subterranean termite (*Reticulitermes flavipes*), the western subterranean termite (*R. hesperus*), and the Formosan subterranean termite (*Coptotermes formosanus*) (Carey 2001; Jenkins et al. 2007; Korb and Hartfelder 2008). Subterranean termites are by far the most economically important family of termites (Table 2-2). With eighty percent of subterranean termites considered to be among economically important pests in the world (Su et al. 2000). The genus *Coptotermes* is a worldwide pest termite and has more economic impact than all other termite species found in the world (Baker and Bellamy 2006). In addition, they are the most destructive and economically important insect pest of wood and other cellulose products and they are responsible for 80% of all termite damage (Su and Scheffrahn 1990; Baker and Bellamy 2006). Their cryptic (Su and Puche 2003; Su 2005) and subterranean natures make them more difficult to control. Control and repair costs due to Formosan subterranean termites in the United States, for example, have been estimated to be more than one billion dollars annually (Culliney and Grace 2000; Lax and Osbrink 2003). Subterranean termites damage about 10–30 percent of harvested kernels of groundnut in Mali, Burkina-Faso, Niger, and Nigeria (Umeh and Ivbijaro 1999; Obi et al. 2008). In India, they are responsible for the loss of 15–25% of maize yield and about 1478 million Rupees (Joshi et al. 2005).

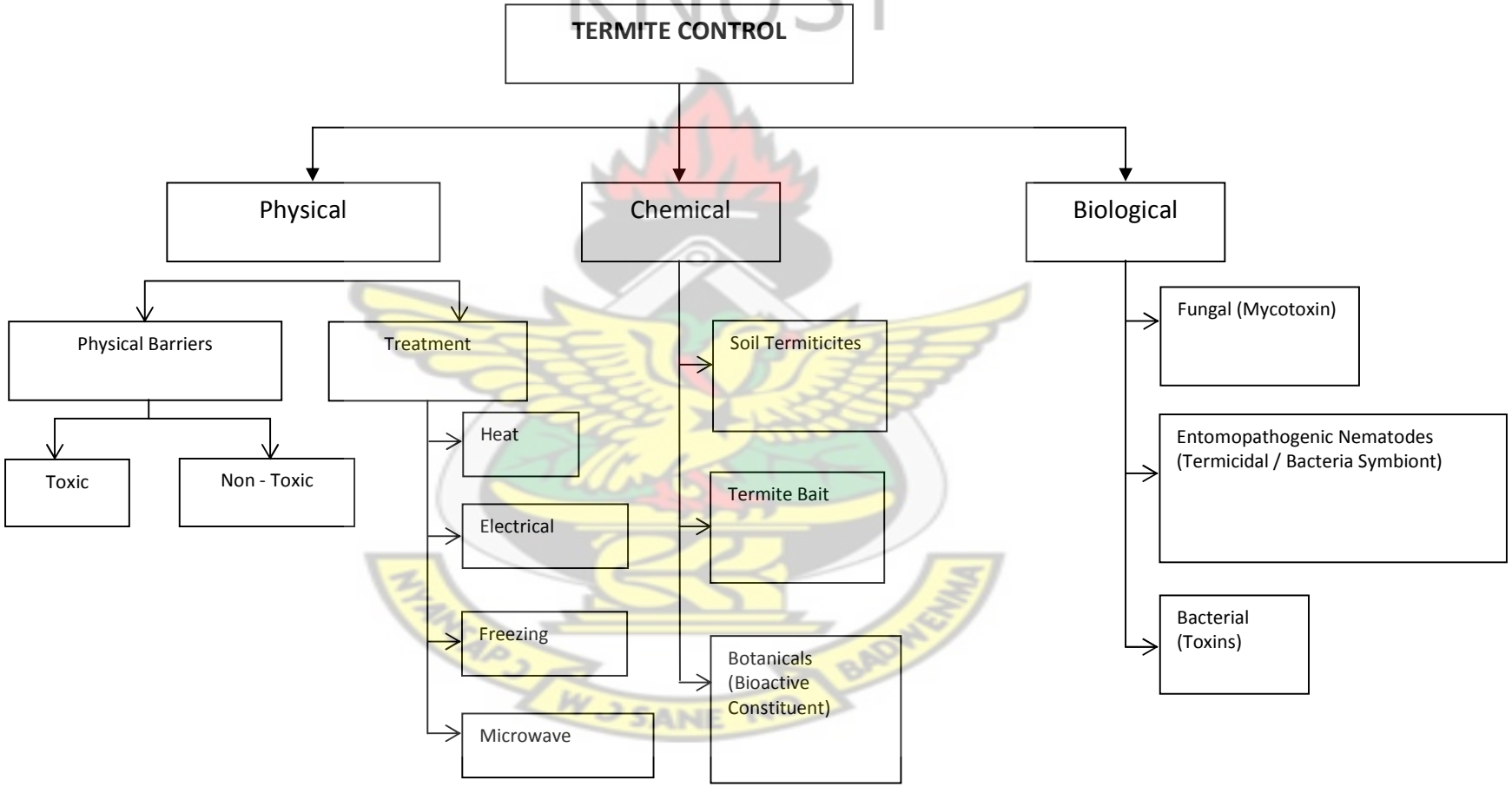
Table 2-2: Economic losses attributable to termite activites

Country	Crop losses (%)	Building damage (%)	Economic losses/annum (Million US $)	Reference/Source
Australia	–	–	>95.24	(UNEP 2000)
Brazil	–	42.7	–	(Milano and Fontes 2002)
China	–	80–90	248.68–292.79	(Zhong and Liug 2002)
Europe	–	–	313	(UNEP 2000)
India	15–25 (Maize crop)	–	35.12	(Joshi et al. 2005)
Japan	–	–	800	(UNEP 2000)
Malaysia	–	70 – Residential 20 – Industrial 10 – Commercial	8–10	(Lee 2002)
Southern Africa	3–100	–	–	(Mitchell 2002)
Spain	–	53.2	–	(Gaju et al. 2002)
United States	–	–	>1000	(UNEP 2000)

2.1.3 CONTROL OF TERMITES

Termite control strategies include the use of wood preservatives, physical barriers, application of liquid termiticides for preventive or remedial control, and the use of baits. It has been suggested that the history of wood treatment is as long as the use of wood itself (Richardson et al. 1989). Compounds used included pitch, olive oil and tar. However, health concerns have caused a reduction and cessation in the use of harmful products including coal tar creosote and arsenic products in parts of the world. Wood preservatives in current use include Copper naphthenate and borates. Copper naphthenate was first used as a wood preservative in Germany in 1889, but commercial use of the product began in 1911. Borates are inorganic minerals mined from naturally formed deposits in the earth. They are toxic to many species of wood-destroying insects and fungi. These compounds maintain their preservative properties for extended periods when they are not rewetted constantly (Davis 2003; Annis 2004; Fleurat-Lessard 2004). Known termite control methods used worldwide are summarised in Figure 2-1 with the important ones discussed below.

Figure 2-1: A schematic classification of major termite control measures (adapted from (Verma et al. 2009))



2.1.3.1 PHYSICAL METHODS

Physical barriers are a very popular method of preventing subterranean termite attack on wooden structures. Physical barriers include concrete slabs, graded particles such as sand, crushed rock, granites and basalts, glass and solid sheet material including high-grade stainless steel. Marine-grade aluminium, certain plastics and woven stainless steel mesh are also used (UNEP 1992; Su et al. 2004; Mulrooney et al. 2007).

These materials act as mechanical barriers to prevent termite penetration and damage to buildings. The use of graded materials including sand, crushed rock, granites and basalts, glass and stainless steel mesh is based on the principle that certain sizes are too small for termites to pass between and too large to be picked up in termite jaws and used to build tunnels. For example, at least 50% of the particles of coarse sand are between 1.4 and 2.8 mm and no more than 25% of the mixture is smaller than 1.4 mm which makes it an effective physical barrier. Similarly, the holes in a stainless mesh are too small for a termite to pass through with the mesh too large for a termite to bite through. These materials may also be impregnated with chemical termiticides to create a toxic zone as well as physical barrier around the structure to prevent termites from gaining access from the ground (Ewart 2000).

Other physical methods include heat, cold, electricity, and microwaves. Heat treatment is an alternative to chemical fumigation for complete building treatment of drywood termites. The use of heat is informed by the fact that termites are more attracted to steam-treated wood than to dry-heated wood, as steam-treated wood produced some feeding attractants (Scheffrahn et al. 1997; Doi et al. 1999). The termites are killed by the

heat generated as they get attracted to steam treated wood. Electrical treatment involves electrocuting termite infested wooden material by passing a high voltage electrical shock of low current at high frequency through the wood and termite galleries to kill the termites (Lewis and Haverty 2000; Myles 2005). Cold treatment involves pumping liquid nitrogen into the infested area and chilling it down to about 20^{o} F below freezing. This effectively freezes the termites but the method may not be applied in large areas, as it can shatter window glasses (Verma et al. 2009).

2.1.3.2 CHEMICAL METHODS

Chemical treatment methods are the most widely used to provide chemical barriers to entry of subterranean termites into structures and reduce the infestation of termites. The methods include the application of chemicals to soil surrounding and under buildings at pre or post construction stages, application of chemicals to wood and use of baits.

The use of chemical compounds to control subterranean termites was suggested at the latter part of the 19^{th} century, but actual evaluation of candidate compounds began in the 1940s. Compounds used included calcium cyanide, sodium cyanide, and carbon disulfide. Chlordane, considered a toxic soil barrier termiticide (Thorne and Traniello 2003; Mulrooney et al. 2007), came into use in 1952 after years of efficacy tests. Chlordane and other cyclodienes; heptachlor, aldrin, and dieldrin became the preferred agents for control of subterranean termites into the 1980s. These repellent compounds function by repelling worker termites from tunnelling toward the foundation of the structures (Su et al. 2003) and were very effective when applied correctly. Their persistence in the environment and the resultant public health concerns however, led to their withdrawal

from the market in 1988. This necessitated a shift to organophosphates such as chlorpyrifos. Organophosphates, even though less persistent than the cyclodienes were more toxic to vertebrates and this led to their ban by the EPA in 2000. Several termiticides containing ingredients listed in Table 2-3 became popular around the world under various brand names and replaced the organophosphates. These termiticides are generally categorised as repellent or non-repellent based on how they affect tunnelling behaviour of termites. Their efficacy as termiticides depends on their chemical toxicity, formulation and application method, as well as termite behaviour and gallery system architecture (Scheffrahn et al. 2001). All the synthetic chemicals containing bifenthrin, cypermethrin permethrin and fenvalerate classified as repellent termiticides are pyrethroids. Pyrethroids have a relatively long residual life, are effective at low use rates, and have low mammalian toxicity and are generally less persistent but more expensive (Baker and Bellamy 2006; Mulrooney et al. 2007; Cookson et al. 2009). Treatment of structures with repellent compounds was rigorous mainly because of the need to eliminate gaps or untreated regions that easily become highways by which termites enter and damage structures. Remedial control with repellent compounds was also complicated by the ability of termites to detect, seal off, or otherwise avoid the treated sections of the colony (Narins et al. 1997; Sumpter 2006; Inta et al. 2007). Environmental toxicity and harmful effects of repellent compounds on non-target organisms (Hirai and Tomokuni 1993; Gamo et al. 1995; Colt et al. 2004; Alegria et al. 2006; Colt et al. 2006) as well as the need for alternative compounds that were effective at low use rates provided the impetus to develop and use non-repellent compounds and baits.

Table 2-3: - List of some chemicals used in products meant for controlling termites (HDRA 2001)

Alpha-cyperrmethrin	A member of the pyrethroid class of chemicals which are synthetic analogues of the naturally occurring pyrethrums; it is used to form a barrier to repel or kill termites (see also deltamethrin, bifenthrin and permethrin).
Deltamethrin	A synthetic pyrethroid similar to alpha-cypermethrin (see above); it is used in some termiticide products.
Bifenthrin	Another member of the pyrethroid class of chemicals; it is used to form a barrier to repel or kill termites.
Permerthrin	Another synthetic pyrethroid, pyrethrin is commonly used as a barrier to repel or kill termites, and is also used for treatment of timber.
Chlorpyrifos	A member of the organophosphorus class of chemicals that is used as a barrier to repel/kill termites.
Hexaflumuron	A member of the benzoylurea class of chemicals that inhibit chitin formation in insects. It is used in strategically placed bait stations to attract foraging termites, which transfer the chemical throughout the colony.
Triflumuron	Another benzoylurea insecticide, triflumuron is applied directly to termite nests.
Imidacloprid	A member of the relatively new class of chemicals called chloronicotinyls. It is used to create a barrier or treated zone in the soil where it attracts termites, which die within the treated zone (partly from the effect of the chemical and partly from infection with fungi and other soil microorganisms).
Fipronil	An extremely active insecticide belonging to the phenylpyrazole family, which has also been developed relatively recently. It is applied by spraying, trenching and soil rodding as a chemical soil barrier around existing structures, and may also be used to protect poles and fence posts.
Arsenic trioxide	A compound used to directly kill termites in active passages (this method has variable effectiveness).

Non-repellent compounds are toxic but usually slow-acting compounds that can be applied as liquid treatments or formulated as baits. Compounds containing fipronil, imidacloprid, and chlorfenapyr became popular in the United States, at the expense of their repellent counterparts (Shelton and Grace 2003) and accounted for about 60% of the total amount of termiticides used in 2002. Application of liquid termiticides involves trenching around the perimeter of a structure and/or drilling holes at regular intervals into the foundation block and slabs (Ying and Kookana 2006). Trenches are filled with finished solution at a rate of 15141.65 cm^3 per 304.8 cm (linear distance) per 30.48 cm of depth to the footer (Ying and Kookana 2006).

Both repellent and non-repellent termiticides have proven satisfactory for making effective barriers when applied properly. The integrity of the soil treatment is a key factor in providing protection to the structure. A repellent termiticide properly applied to the soil will provide protection to the structure unless the barrier is disturbed. A termiticide barrier composed of a non-repellent termiticide allows more flexibility and will provide protection even if the integrity of the barrier is disturbed. Repellent termiticides often do not kill termites because they are able to detect the chemical treatment and so they do not tunnel into the treated soil or structure (Koehler and Tucker August, 2003). On the other hand non-repellent termiticides do not affect termite tunnelling because the termites are unable to detect the treated soil or structure. So they continue to tunnel freely through the treated soil and structure and become exposed to the non repellent termiticide by contact or ingestion and eventually die (Koehler and Tucker August, 2003).

PRECONSTRUCTION AND POST-CONSTRUCTION CHEMICAL APPLICATIONS

The control of termites in buildings is achieved by preventing termites from entering buildings at beginning of construction or in most cases after construction. Before the concrete is poured the soil underneath and surrounding the concrete slab is treated with a chemical termiticide. In addition the termiticide is applied to both the inside and outside of the foundation and also around piers, chimney bases, pipes, conduits and any other structures that come in contact with the soil (Bach, 1990).

A thorough pre-construction treatment of soil and structures in contact with the soil should protect the structure for at least 5 years. For example houses treated prior to 1988 with chlorinated hydrocarbons, such as chlordane or heptachlor, should be protected from termites for 30-40 years (Ewart, 2000; Hirai and Tomokuni, 1993).

CHEMICAL TREATMENT OF WOOD

Chemical treatment of wood increases their termite resistance and ability to prevent termite attack and it is a common and effective method of termite control (Johnston et al. 1971). Wood treated with disodium octaborate tetrahydrate induced high termite mortality (Lu et al. 2008). Chromated copper arsenate (CCA) was commonly used as a wood preservative against termites, but due to its negative environmental effects it is formulated as copper borate, water-borne copper naphthanate, and N^{0} N-naphthaloylhydroxylamine. Nowadays, multi-component biocide systems combining a borate base supplemented with either 0.1% azole or 0.5% thujaplicin are being used. They are nontoxic, non volatile, odourless, hypoallergenic and able to provide long-term protection (Clausen and Yang 2007).

BAITING TECHNOLOGY

Baiting is the most recent method of termite control. It is environmentally sound and utilizes very small amounts of insect toxicants. In baiting technology termite colonies can be eliminated by the use of toxic or nontoxic baits. Bait is a wood or a cellulose matrix favoured by termites that is impregnated with a slow-acting toxic chemical or nontoxic substance such as fungal spores, mycelium (that grows through termite cuticle and utilizes entire termite body) and infective stages of nematodes (which carry bacterium which produces toxins lethal to termites) (Evans 2001; Evans and Gleeson 2006; Huang et al. 2006).

Bait stations are placed into soil at intervals around the building. Termite workers feed upon the bait and transfer the toxicant to other colony members by grooming or trophallaxis, eventually reducing or eliminating the entire colony. Bait consumption by termites depends on bait design, with termites preferring larger baits over smaller ones (Evans 2001; Evans and Gleeson 2006; Huang et al. 2006). Termites are not site-specific, but rather, they forage among various food sites, which results in the bait being encountered by many colony members. The toxicant must be slow acting because termites tend to avoid sites where sick and dead termites accumulate. Successful termite baiting needs proper monitoring and maintenance of the stations. Baits are often used in sensitive environments. Commercially available baits for termite control contain ingredients such as diflubenzuron, chlorflurazuron, hexaflumuron, triflumuron, sulfluramid, noviflumuron, disodium octoborate tetrahydrate, arsenic trioxide, fipronil and hydramethylnon. Those containing hexaflumuron are the most potent bait toxicants (Sajap et al. 2000; Osbrink et al. 2005; Su 2005).

Bait units require regular inspections to check the untreated cellulose component for termites which when present necessitate the replacement of the cellulose with bait compound (Evans and Gleeson 2006). The performance of baits is, however, compromised by the presence of competing food sources such as the structure and natural food sources near it (Jones 2003; Haagsma and Rust 2005; Sukartana et al. 2009).

BOTANICALS

The use of chemical control is a proven means of protection from termites but the excessive use of chemicals is a serious environmental concern as target insects develop resistance (Kamble et al. 1992; Mulrooney et al. 2006). So the search for new methods of termite control initiated in 1935 (Trikojus 1935) is ongoing and biological methods could be suitable alternatives in this regard. Biological methods include the use of plant derived products and parts such as essential oils, seeds, bark, leaves, fruits, roots, wood and resins and, deployment of entomopathogenic fungi, nematodes and bacteria against termites (Meepagala et al. 2006; Verma and Verma 2006; Seo et al. 2009).

Anti-termitic capabilities of plant-derived products

Various plant-derived products and parts are known to possess antitermitic properties, including termiticidal activity, repellency, antifeedance and insect growth regulation. The antitermitic activity exhibited depends on the type of plant and which products or part (essential oil, seed, bark, leaf, fruit, root, wood and resins) is being applied (Verma et al. 2009).

The insecticidal activity of essential oils was evaluated as early as 1972 (Nakashima and Shimizu 1972). Various essential oils have been evaluated for repellency and toxicity

against termites (Zhu et al. 2001a). Vetiver oil has long-lasting activity, and has been proven the most effective (Zhu et al. 2001b). Nootkatone, a sesquiterpene ketone component is responsible for the strong repellent, feeding deterrence and toxicant effects of vertiver grass oil on Formosan subterranean termites (Maistrello et al. 2001; Zhu et al. 2001b). Nootkatone negatively affects termites for 12 months and is more long-lasting than vetiver oil (Maistrello et al. 2003). Nootkatone acts as a feeding deterrent that results in almost a complete loss of Pseudotrichonympha grassii koidzumi, the most important flagellate species for cellulose digestion in the Formosan subterranean termite (Maistrello et al. 2001). Vetiver oil and nootkatone can be used as novel pesticides that can be incorporated into potting media for substrate (soil, wood, and mulch) treatments to reduce the spread of Formosan subterranean termites (Mao and Henderson 2007). A field evaluation of vetiver grass root mulch treatment showed decreased tunnelling activity and wood consumption and increased mortality of Formosan subterranean termites. Vetiver oil can be chemically modified to enrich sesquiterpenones and other structurally related compounds exhibiting potent insecticidal activity (Chauhan and Raina 2006).

Table 2-4: Some effects of essential oils of various plants on termites (Verma et al. 2009)

Plant	Part	Active component	Activity
Thujopsis dolabrata Siebold & Zucc.	Wood	b-Thujaplicin and carvacrol	Toxic
Chamaecyparis pisifera (Siebold & Zucc.) Endl.	Wood	Chamaecynone and isochamaecynone	Toxic
Cryptomeria japonica D.Don	Wood	b-Eudesmol and cedrol	Toxic
Azadirachta indica A.Juss.	Seed	Limonoids	Antifeedant
Chamaecyparis obtusa Siebold & Zucc.	Wood	Monoterpene, sesquiterpene, and sesquiterpene alcohol	Toxic
Thujopsis dolabrata Siebold & Zucc.	Wood	Thujopsene	Toxic
Taiwania cryptomerioides Hayata	Wood	Cedrol and a-cadinol	Toxic
Vetiveria zizanioides Nash	Root	Nootkatone (a sesquiterpene alcohol) and cedrene	Arrestants, feeding deterrent, repellent and toxic
Cinnamomum osmophloeum Kaneh.	Leaf	Cinnamaldehyde	Toxic
Tagetes erecta L.	Leaf	(Z)-ocimene	Mortality
Lepidium meyenii Walp.	Leaf	Benzylthiocynate, 3-methoxyphenylacetonitrile and b-ionone	Feeding deterrent
Melaleuca gelam and Melaleuca cajuputi Powell	Leaves and twigs	Elemene, g-terpinene and terpinolene, Monoterpenes, sesquiterpenes, hydrocarbons and a diterpene	Toxic
Calocedrus formosana (Florin) Florin	Leaf	T-muurolol	Toxic
Allium sativum **L.** and *Eugenia caryophyllata* Thunb.	Bud	Diallyl trisulphide, Diallyl disulphide, eugenol, Diallyl sulfide and b-caryophyllene	Toxic
Callitris glaucophylla Joy Thomps. & L.A.S.Johnson	Wood	Guaiol, a-eudesmol, and b-eudesmol, citronellic acid and geranic acid	Repellent

The leaf essential oil of *Tagetes erecta* L. rich in (Z)--ocimene (42.2%) showed significant termiticidal activity. Complete mortality of *O. obesus* Rhamb was observed at a dose of 6 ml/petri-plate of leaf essential oil after24 hours of exposure (Singh et al. 2002). Essential oils of aerial parts of Maca, *Lepidium meyenii* Walp act as a feeding deterrent to termites. Minor components 3-methoxyphenylacetonitrile and benzylthiocyanate showed good activity against Formosan subterranean termites (Tellez et al. 2002). The essential oil of catnip, *Nepeta cataria* (lamiaceae) acts as a barrier to the subterranean termites *R. flavipes* (Kollar) and *R. virginicus* (Banks) (Peterson and Ems-Wilson 2003). *Calocedrus formosana* shiraki leaf essential oil and its main constituent, T-muurolol, caused 100% mortality of *C. formosanus* at the dosage of 5mg/g (Cheng et al. 2004). Essential oils from three species of coniferous tree have significant antitermitic activity against C. formosanus shiraki. The results demonstrated that at the dosage of 10 mg/g, the heartwood and sapwood essential oils of *Calocedrus macrolepis* var. formosana and *Cryptomeria japonica* and the leaf essential oil of *Chamaecyparis obtusa* var. formosana had 100% mortality after 5 days of test. Among the tested essential oils, the heartwood essential oil of *C. macrolepis* var. formosana killed all termites after 1 day of test, with an LC(50) value of 2.6 mg/g, exhibiting the strongest termiticidal property (Cheng et al. 2007). Leaf essential oil from two *Melauleuca* species, gelam and cajupati, were tested for their termiticidal activity. Gelam oils were rich in compounds with a high boiling point and which separated into the elemene-rich type and g-terpinene and terpinolene types. Cajupati oils were characterized into three chemotypes according to their 1, 8 cineole content; as high, low or none. Gelam oils were found to be more effective than cajupati oils (Sakasegawa et al. 2003). In a study, essential oils from 29 plant species demonstrated a significant

insecticidal activity against the Japanese termite, *Reticulitermes speratus* Kolbe with essential oils of 19 species including clove bud and garlic applied at 7.6 microL/L of air (Park and Shin 2005). Over 90% mortality after 3 days was achieved with *O. japonica* essential oil at 3.5 microL/L of air. *Eucalyptus citriodora* Hook*, Cinnamomum cassia* Nees ex Blume*, Allium cepa* L*, Illicium verum* Hoof f*, Stephanomeria tenuifolia* Goodrich and S.L. Welsh*, C. roborowskii*, clove bud, and garlic oils at 3.5 microL/L of air were highly toxic 1 day after treatment. At 2.0 microL/L of air concentration, essential oils of I*. verum, C. roborowskik, S. tenuifolia, A. cepa* L, clove bud, and garlic gave 100% mortality within 2 days of treatment. Clove bud and garlic oils showed the most potent antitermitic activity among the plant essential oils.

Different parts of a number of plants, such as leaf, flower, fruit, and root, contain some bioactive components and can be extracted and used as termite control agents as shown in Table 5. These extracts may act to kill microbes found in the hindgut of Formosan subterranean termites (Ohkuma et al. 2000; Doolittle et al. 2007), as deterrants (Cornelius et al. 1997) or as antifeedants against termites (Ohmura et al. 2000; Boue and Raina 2003).

Hexane and methanol extract of leaves of Juniperus species have shown termiticidal activities (Adams et al. 1988). A neem insecticide formulation, Margosan-O, containing 0.3% azadirachtin and 14% neem oil, was toxic against the Formosan subterranean termite (Grace and Yates 1992). *Detarium microcarpum* Guill and Perr possessed strong antifeedant activity when the methanol extract of its leaves was tested against termites. Four clerodane diterpenesd3, 13E-clerodien-15-oic acid; 4(18), 13E-clerodien-15oic acid;

18-oxo-3,13E-clerodien-15-oic acid; and 2-oxo-3,13Eclerodien-15-oic acid were isolated and found to be effective at a concentration of 1%(Lajide et al. 1995). *Acorus calamus* L rhizomes and aerial parts of *Tagetes erecta* Linn. were found to be toxic against *O.obesus* (Sharma et al. 1999). Hexanes, diethylether, and ethanol fractions of tarbush (*Flourensia cernua* DC) leaves exhibited a high degree of antitermite activity. The hexane fraction contained mostly monoterpenoids, while the ethanol fraction volatiles were primarily sesquiterpenoids.

Four Echinops species of 220 crude extracts of plants native to Greece and Kazakhstan tested were found to be active against termites (Fokialakis et al. 2006). Eight thiophenes were further isolated and tested, with results showing varying degrees of termiticidal activity. Two compounds, terthiophene and bithiophene, demonstrated 100% termite mortality within 9 days against the Formosan subterranean termites. Soil treated with seeds of *Withania somnifera* Dunal, *Croton tiglium* L, and *Hygrophila* auriculata (Schumach) Heine disrupted the bacterial activities in the gut of *Microtermes obesi* Rambur. Seed extracts of *W. somnifera* and *H. auriculata* were highly toxic in a 6 day period. Areas of tunnelling and the number of bacterial colonies were also reduced at 100% concentration of *W. somnifera* and H. auriculata (Ahmed et al. 2000). Lantana camara var. aculeata leaves were studied for their termiticidal effects. A 5% chloroform extract was most effective (Verma and Verma 2006).

Quinones isolated from the chloroform extract of the roots of *Diospyros sylvatica* Roxb were found to be toxic against *O. obesus*. The major termiticidal components identified were plumbagin, isodiospyrin and microphyllone (Ganapaty et al. 2004). Hexane extract of

Xylopia aethiopica (Dunal) A. Rich fruits and aqueous methanol extract of the seeds were studied for their antifeedant activity against R. speratus workers. The crude extract at 1% concentration exhibited strong antifeedant activity and out of the six ent-kaurane diterpenes isolated, (-) – Kaur – 16- en-19-oic acid had the strongest antifeedent activity (Lajide et al. 1995).

As shown in Tables 2-4 and 2-5, some plants and trees are resistant to termite attack due to the presence of some active components as part of their natural defence comparable to that of commercial wood preservatives (Onuorah 2000; Verma et al. 2009). For example all taxa of Juniperus examined in the US exhibited termiticidal activities for the fresh heartwood sawdusts. Both hexane and methanol (sequential) extracts of the heartwoods bark/sapwood and leaves investigated for termiticidal activities showed termiticidal activities (Adams et al. 1988; Arango et al. 2006). Four antitermitic compounds decatalponol, epicatalponol, catalponone, and catapalactone were isolated from *Catalpa bignonioides* Walter heartwood with catalponol and catapalactone being the most effective against *R. flavipes*. Resins obtained from plants and trees are also known to exhibit resistance to attacks from other insects (Fang and Casida 1999; Birkett et al. 2008).

Table 2-5: Some effects of plant extracts on termites (Verma et al. 2009)

PLANT	PART	ACTIVE COMPONENT	ACTIVITY
Adina racemosa Miq.	Bark	Benzoic acid	Toxic
Aframomum melegueta K.Schum.	Seed	Gingerol [5-hydroxy-L-(4-hydroxy- 3-methoxyphenyl)decan-3-one] and shogaol [1-(4-hydroxy-3-methoxyphenyl)dec-5-en-3-one]	Antifeedant
Detarium microcarpum Guill. & Perr.	Leaves	Clerodane diterpenes, 3,13E-clerodien-15-oic acid, 4(18),13E-clerodien-15-oic acid, 18-oxo-3,13E-clerodien-15-oic acid and 2-oxo-3,13E-clerodien-15-oic acid	Antifeedant
Xylopia aethiopica A.Rich.	Fruits and seeds	Diterpenes and amides	Antifeedant
Moneses uniflora A.Gray	Aerial parts	Naphthoquinones, 2, 7-dimethyl-1,4-naphthoquinone and 3-hydroxy-2,7-dimethyl-1,4-naphthoquinone	Toxic
Flourensia cernua DC.	Leaves	Monoterpenes and sesquiterpenes	Toxic
Diospyros sylvatica Roxb.	Root	2-methyl-anthraquinone, plumbagin, diosindigo, isodiospyrin and microphyllone (quinones)	Repellant and toxic

Table 2-6: Some effects of wood extracts of trees on termites (Verma et al. 2009)

PLANT	ACTIVE COMPONENT	EFFECT ON TERMITES
Kalopanax septemlobus Koidz.	Saponins	Toxic
Ternstroemia japonica Thunb.	Barrigenol glycoside (saponin)	Toxic
Podocarpus macrophyllus D.Don	Inumakilactone (bisnorterpenoid)	Toxic
Callistris species	L-citronellic acid, D-citronellic acid, L-dihydrocitronellic acid, D-dihydrocitronellic acid, geranic acid, tetrahydrogeranic acid, caprylic acid, pelargonic acid and enanthic acid	Lethal
Pinus lambertiana Douglas	Fatty acids and alpha halogenated compounds	Feeding deterrent
Sciadopitys verticillata Siebold & Zucc.	Isoeugenol mono-Me ether and cedrol	Toxic
Chamaecyparis obtusa Siebold & Zucc.	Diterpenes, T-muurolol and a-cadinol	Toxic
Chamaecyparis lawsoniana (A. Murray) Parl.	a´ -terpineol and 3sesquiterpene alcs., T-cadinol, torreyol (a¨-cadinol), and a´ -cadinol	Toxic
Pometia pinnata J.R.Forst. & G.Forst.	Saponins	Toxic
Catalpa bignonioides Walter	Catalponol and catalpalactone	Toxic

Table 2-7: Some effects of resins from plants/trees on termites (Verma et al. 2009)

PLANT	TERMITE SPECIES	EFFECT
Dipterocarpus kerrii King	Zootermopsis Angusticollus (Hagen)	Toxic
Dipterocarpus kerrii King	Neotermes	Toxic
Parthenium argentatum A.Gray	C. formosanus And Heterotermes sp.	Repellant and antifeedant
Dipterocarpus kerrii King	Neotermes dalbergiae	Toxic
Parthenium argentatum A.Gray *Parthenium tomentosum* DC. and *Castela emoryi* (A.Gray) Moran & Felger	R. flavipes	Antifeedant
Parthenium argentatum A.Gray	Reticulitermes spp.	Toxic

2.1.3.3 BIOLOGICAL

Pathogenic organisms such as entomopathogenic fungi, bacteria and nematodes (round worms) are used as biological control agents. These agents infest and kill termites and other soil insects.

BIOLOGICAL CONTROL OF TERMITES USING NEMATODES

Two families of nematode (Phylum Nematoda), *Steinernematidae* and *Heterorhabditidae*, are obligate insect parasites and are associated with bacterial symbionts Xenorhabdus spp. and Photorhabdus spp. (Boemere et al. 1993; Forst et al. 1997). They are widely used in biological control of termites (Massey 1971; Yu et al. 2006). The infective stage of the nematode is free-living in the soil and infects the termite making it release symbiotic bacteria into the termite hemocoel, causing septicemia and death (Wilson-Rich et al. 2007). However, it seems that the outcome of nematode control depends on the termite and nematode species respectively as results of laboratory experiments based on this knowledge are not consistent. High mortality was observed with nematode infestation of *R. flavipes* but experiments with termite species *R. tibialis* (Epsky and Capinera 1988) and *C. formosanus* were not successful. A study to record mortality of the subterranean termite *Heterotermes aureus* using *Heterorhabdidtis bacteriophora* and *Steinernema carpocapsae*, showed *S. carpocapsae* to be more potent in causing termite *H. aureus* mortality than *H. bacteriophora* (Verma et al. 2009).

BIOLOGICAL CONTROL OF TERMITES USING BACTERIA

Some rhizobacterial species are known to produce and excrete hydrogen cyanide (HCN) into the rhizophere. HCN-producing rhizobacteria could be useful for termite control if introduced into termite mounds, thereby localizing cyanide production and minimizing potential deleterious effects on other soil fauna. Release of HCN by rhizospheric bacteria into the soil can be toxic to subterranean animals. For example, HCN-producing Pseudomonas aeruginosa has been shown to have lethal effects on nematodes (Darby et al. 1999; Gallagher and Manoil 2001). Non-parasitic rhizobacteria that produce harmful metabolites might also facilitate the biocontrol of termites and might be an alternative to chemical control of termites. Three different species of HCN-producing rhizobacteria, *Rhizobium radiobacter, Alcaligenes latus*, and *Aeromonsa caviae* were found to be effective in killing the *O. obesus* termites under laboratory conditions (Devi et al. 2006).

BIOLOGICAL CONTROL OF TERMITES USING FUNGI

Approximately 750 species (56 genera) of fungi have been isolated from insects, many of which offer great potential for pest management. Biological control with pathogenic fungi seems a promising alternative to chemical control of termites. The pathogenicity of a fungus toward insects is dependent upon a complex relationship between the ability of the fungus to germinate on the cuticle, its ability to penetrate the cuticle, and the ability of the insect's immune system to prevent fungus growth. However, strains of fungi pathogenic for one particular host species may not show the same growth characteristics and pathogenicity in another insect species (Cornelius et al. 2002; Torres et al. 2004; Yanagawa et al. 2008).

2.1.4 PHYTOCHEMICALS

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are secondary metabolites and include glycosides, alkaloids, carotenoids, terpenoids, saponins, coumarins and antraquinones. These chemicals are produced by plants to protect themselves but have been shown to protect humans against diseases and pests. Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Cowan 1999; Gibbons 2005; Verma and Verma 2006).

2.1.4.1 GLYCOSIDES

Glycosides is a general term covering a wide range of substances whose common feature is that they consists of at least one sugar molecule linked via its anomeric carbon to another moiety. Glycosides are classified according to the structure of the aglycone including anthracene derivative, flavonoid, cardenolide and cyanogenic glycosides (Chandler 1985; IUPAC 1997). The aglycones are released from the glucosides by hydrolysis and the phamarcological activity is found in the aglycone part (Fig 2-2).

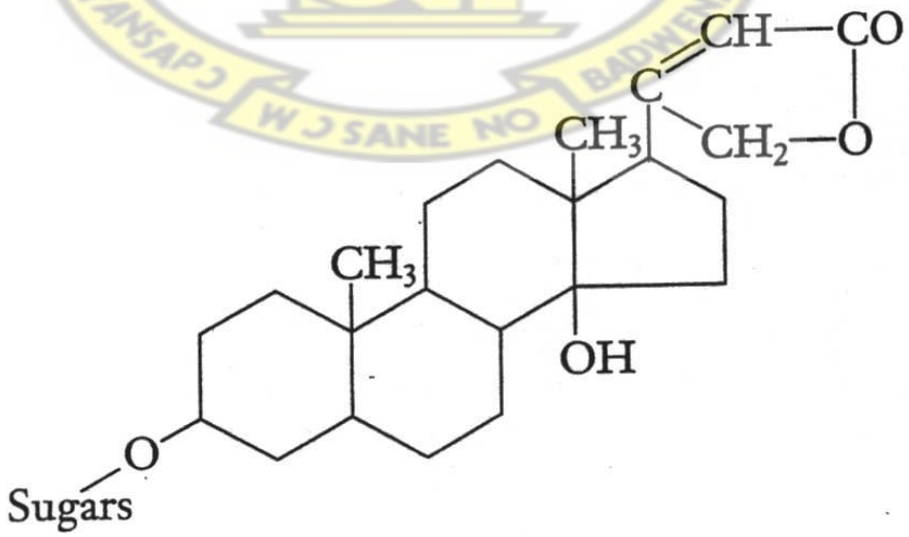


Figure 2-2: Chemical structure of glycosides

Glycosides are relatively polar due to the presence of one or more sugars in the molecule. Most glycosides can be extracted with polar solvents such as acetone, ethanol, methanol, water or a mix of these (Fiamegos et al. 2004). However cardiac glycosides with their bulky steroidal aglycone have appreciable solubility in chloroform. When water is used for extraction, there is a possibility of enzymic breakdown of the glycosides to aglycones by the action of glycosidase co-extracted from the plant material. However, this is prevented if boiling water is used or if a significant proportion of alcohol or ammonium sulphates are added to the extract. In plants glycosides serve several purposes including defense and prevention of decay of damaged tissues.

Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residues (Mabry and Ulubelen 1980). The hydroxyl groups are nearly always found in positions 5 and 7 in ring A, while ring B commonly carries hydroxyl or alkoxyl groups at the 4^1 – position, or at both 3^1- and 4^1-positions. The flavonoid compounds can be regarded as C_6-C_3-C_6 compounds, in which each C_6 moiety is a benzene ring. The variation in the state of oxidation of the connecting C_3 moiety determines the properties and class of each compound (Jangaard 1970; Guchu et al. 2007). Flavonoids occur in all parts of plants, including the fruit, pollen, roots and heartwood. Numerous physiological activities have been attributed to them. Condensed flavonoids, both flavano-tannins and polycyclic flavanoids like peltogynols in various plants are found to be the insecticidal principles (Abe et al. 1995; Ohmura et al. 2000; Boue and Raina 2003; Benavides et al. 2007).

2.1.4.2 ALKALOIDS

All alkaloids contain at least one nitrogen atom and in the majority of cases the compound is basic. Salt formation can occur in the presence of an acid. In their extraction, the plant materials are either basified using diethylmine or ammonia and extracted with an organic solvent (Hultin 1966; Macabeo et al. 2005). The alkaline medium ensures the alkaloids are in their free base or unionized state. Most alkaloids are of medium polarity and can be extracted using chloroform, dichloromethane or diethylether. Ethanol, a general solvent may also be used. The plant material can also be treated with aqueous acid forming salts which are ionized and so are soluble in aqueous media. The alkaloid is then recovered in free base form by basifying the aqueous extracts (which depronates the alkaloid). This is then extracted into a suitable organic solvent. Alkaloids have a range of antimicrobial and insecticidal properties including antifeedant and toxic properties and have potential for commercial development as wood treatment agents (Yang et al. 2002; Kim and Mullin 2003; Mao and Henderson 2007).

2.1.4.3 CAROTENOIDS

Carotenoids are generally tetraterpenoids derivatives containing about 40 carbon atoms. They can be divided into hydrocarbons and oxygenated forms known as xanthiphylls. Hydrocarbons are less polar and can be extracted into petroleum ether. Xanthophylls are more polar as they contain alcohols, ketones, aldehydes, and acid or epoxide groups and can therefore be extracted into ethanol or mixtures of ethanol and less polar solvents such as chloroform (Kitajima et al. 2003). Many naturally occurring substances such as sterols, bile, sex hormones, adrenal cortical hormones, cardiac glycosides, toad poisons

and sapogenins, contain the cyclopentanoperhydrophenanthrene ring system or in very rare cases, a modification of it (El-Agamey et al. 2004).

2.1.4.4 TERPENOIDS

Terpenoids are widely distributed in nature, mostly in the plant kingdom. They may be regarded as derivatives or oligomers of 2-methyl-1, 3-butadiene (isoprene), usually joined head to tail. Several terpenes exert a repellent action on insects. For example, Thujopsene and Cedrene are the insecticidal principles in *Juniperus recrua*. Terpenoids are abundant in essential oils. They consist of a complex mixture of terpenes or sesquiterpenes, alcohol, aldehydes, ketones, acids and esters. Plant essential oils and extracts containing terpenoids show good activity against micro-organisms and pests (Meinwald et al. 1978; Cornelius et al. 1997; Tellez et al. 2001; Sridhar et al. 2005).

2.1.4.5 SAPONINS

Saponins are glycosides with a distinctive foaming characteristic. They are found in many plants, but get their name from the soapwort plant (*Saponaria*), the root of which was used historically as soap. They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C_3 and an ether bond to a sugar side chain (Voutquenne et al. 2005; Gao and Wang 2006). The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water soluble side chain. Saponins are bitter and reduce the palatability of livestock feeds. However if they have a triterpenoid aglycone they may instead have a licorice taste as glucuronic acid replaces sugar in triterpenoids. Some saponins reduce the feed intake and growth rate of non-ruminant animals while others are not very harmful. For example, the saponins found in oats and spinach increase and accelerate the body's ability to absorb calcium and silicon, thus assisting in digestion. Certain pasture weeds contain substantial quantities of dangerous saponins and result in life threatening toxicities for certain animal species (Wickremasinghe and Thirugnanasuntheram 1980; Magalhaes et al. 2003).

2.1.4.6 SAPONIN GLYCOSIDES

Sapogenins are plant glycosides that have the property of forming a soapy lather in water. Below is an example of a sapogenin, the sugar-free moiety of saponin. Saponin glycosides are divided into 2 types based on the chemical structure of their aglycones (sapogenins) (Chandler 1985; IUPAC 1997). Saponins on hydrolysis yield an aglycone known as "sapogenin" (Figure 2-3).

The so-called NEUTRAL saponins are derivatives of STEROIDS with spiroketal side chains. The ACID saponins possess triterpenoid structures.

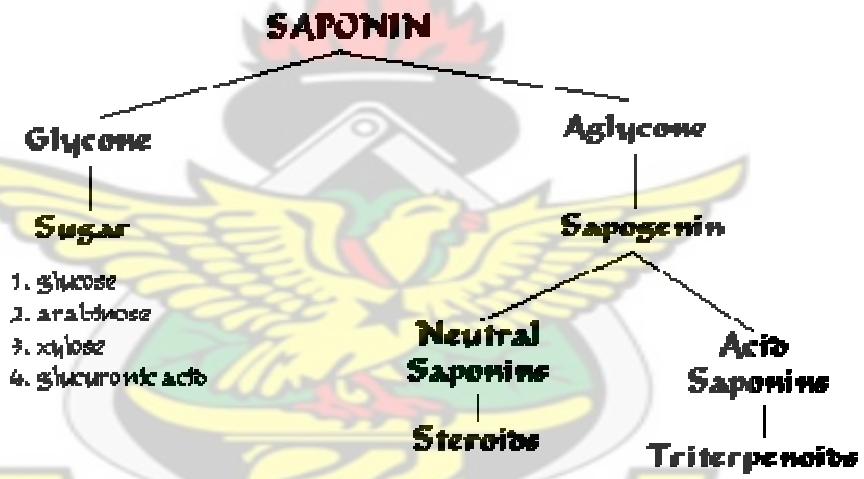
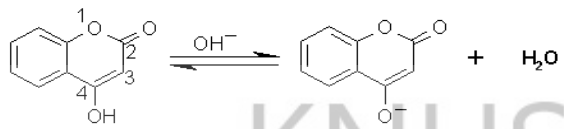


Figure 2-3: Scheme showing constituents of saponins

The main pathway leading to both types of sapogenins is similar and involves the head-to-tail coupling of acetate units. However, a branch occurs, after the formation of the triterpenoid hydrocarbon, squalene, that leads to steroids in one direction and to cyclic triterpenoids in the other (Chandler 1985; IUPAC 1997).

2.1.4.7 COUMARINS

Coumarins and their derivatives are principal oral anticoagulants. Coumarin is water insoluble; however a 4-hydroxy substitution confers weak acidic properties to the molecule that makes it water soluble under slightly alkaline conditions (equation below).



The structures of coumarin and its derivatives are as shown above. Warfarin is marketed as the sodium salt. It has one chiral centre. The S (-) isomer is about 5 - 8 times more potent than the R (+) isomer; however, commercial warfarin is a racemic mixture (Hamdan et al. 2011; He et al. 2011; Wang et al. 2011).

2.1.4.8 ANTHRAQUINONE

Anthraquinone naturally occurs in some plants (e.g. aloe, senna, rhubarb, and Cascara buckthorn), fungi, lichens, and insects, where it serves as a basic skeleton for their pigments. Natural anthraquinone derivatives tend to have laxative effects. Anthraquinone (9, 10-dioxoanthracene) is an aromatic organic compound. Its other names are 9, 10-anthracenedione, anthradione, 9, 10-anthrachinon, anthracene-9, 10-quinone, 9, 10-dihydro-9, 10-dioxoanthracene, and trade names Hoelite, Morkit, Corbit, and others

It is insoluble in water or alcohol, but dissolves in nitrobenzene and aniline. It is chemically fairly stable under normal conditions. There are several ways to obtain anthraquinone.

Some of these are;

1. Oxidation of anthracene

2. Condensation of benzene with phthalic anhydride in presence of $AlCl_3$ (Friedel-Crafts substitution). The resulting o-benzoylbenzoic acid then undergoes cyclization, forming anthraquinone.

3. Diels-Alder reaction (from naphthoquinone and a 1,3-diene)

Anthraquinone condenses with glycerol forming Benzanthrone In this reaction the quinone is first reduced with copper metal in sulfuric acid (converting one ketone group into a methylene group) after which the glycerol is added. Anthraquinone is used in production of dyes, such as alizarin. Many natural pigments are derivatives of anthraquinone. Anthraquinone is also used as a catalyst in production of wood pulp in pulp and paper industry. Another use is as a bird repellant on seeds. A derivative of anthraquinone (2-ethylanthraquinone) is used to produce hydrogen peroxide commercially (Li et al. 2011; Tang et al. 2011; Turcanu et al. 2011; Yildiz et al. 2011).

CHEMICAL CHARACTERISTICS OF ANTITERMITIC COMPOUNDS

A relationship may exist between the chemical structure of a phytochemical compound and its antitermitic property (Scheffrahn and Su 1987). Unhalogenated acids had little effect on C. formosanus mortality and wood consumption as compared to 2-brominated acids, which were significantly, more toxic and resulted in diminished feeding on wood by termites. Methyl esters of haloacids had a variable effect on antitermitic activity that may have been related to carbon-chain length. 2-Iodooctadecanoic acid and ester treatments were more toxic and less fed upon than 2-bromo compounds, which, in turn, were more active than their 2-chloro analogs. Methyl, ethyl, and isopropyl-2-halooctadecanoates were equally or more toxic than their respective haloacids. Noviflumuron (Dow Agrosciences-Recruit III AG Termite bait; $C14_4H_9ClF_9N_2O_3$), bistrifluron ($C_{16}H_7ClF_8N_2O_2$), hexaflumuron (Dow Agrosciences-Recruit AG Termite bait; $C_{16}H_8Cl_2F_6N_2O_3$), and diflubenzuron (Crompton-Dimilin SC 48 Forestry; $C_{17}H_7Cl_2F_2N_2O_3$) are all slow-acting insect toxicants used in termite baits. Noviflumuron is more potent and has faster activity. It caused higher *R. speratus* mortality as compared to haxaflumuron and diflubenzuron (Karr et al. 2004; King et al. 2005). Bistrifluron showed a faster rate of action against *C. formosanus* than hexa-flumuron (Kubota et al. 2006). Hexaflumuron is superior to diflu-benzuron as a bait toxicant against both *C. formosanus* and *R. flavipes* (Su and Scheffrahn 1993).This suggests that the antitermitic activity of these toxicants increases as the number of fluorine molecules increases in their chemical structure. The synthesis of saponins by chemical reactions and also isolating them from *Pometia pinnata* wood to investigate the relationship between chemical structure and antitermitic activity showed that the saponins with two sugar chains had no antitermitic activity while those having a

single sugar chain showed good results (Ohara et al. 1991). Results are the same for naturally isolated saponins. The longer the sugar chains the weaker their antitermite activity. Similarly, the synthesis of triterpenoid saponins (methyloleanolateglycosides) suggested that Methyl oleanolate-3-yl β-D-glucoside and methyl oleanolate-3-yl β -D-cellobioside showed the greatest antifeedant activity with *R. speratus*, and the activity decreased according to the lengthening of the chain of the sugar moiety (Ohmura et al. 1997). Because the molecular hydrophilicity increases with the increasing amounts of sugar residues, it is assumed that adequate polarity is necessary to reveal the antitermitic activities of triterpenoid saponins. These studies suggest that the number of sugar chains, halogenation and carbon-chain length in the chemical structure of the active component are the factors affecting the antitermitic activity (Ohmura et al 1997).

2.2 EXTRACTION, SEPARATION AND INSTRUMENTAL METHODS OF ANALYSIS OF PLANT DERIVED PRODUCTS

2.2.1 EXTRACTION PROCEDURES

To obtain organic constituents from dried plant tissue (heartwood, dried seeds, roots, leaf), it is continuously extracted powdered in a soxhlet apparatus with a range of solvent, starting with non-polar solvent (to separate lipids and terpenoids) and then a polar solvent for more polar compounds such as ether, petroleum, chloroform, alcohol and ethyl acetate. The extract obtained is clarified by filtration and is then concentrated in vacuo (in a rotary evaporator) normally at temperatures ranging between 30^0C and 40^0C (Katz et al. 1966; Salminen 2003; Houtman et al. 2007). The concentrated extract may crystalise on standing. These are normally collected by filtration and their homogeneity tested for by chromatography in several solvents. In the presence of a single substance, the crystals are purified by recrystallisation and further analysis is carried out. With mixture of substances, the crystals are redissolved in suitable solvent and chromatographic methods are used to separate them (Katerere et al. 2004).

2.2.2 SEPARATION OF PLANT DERIVED SUBSTANCES

Chromatography techniques are mainly employed in separation and purification of plant constituents (Wagman and Cooper 1989; Björnstad et al. 2009). Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase. The phases are chosen such that components of the sample have differing solubilities in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very

soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase (Wagman and Cooper 1989; Björnstad et al. 2009).

Chromatography techniques include: paper chromatography (PC), thin layer chromatography (TLC), gas liquid chromatography (GC) and high performance liquid chromatography (HPLC). Chromatography can be used to separate and purify a large variety of substances, from chlorophyll and other plant pigments, through amino acids in cell or tissue samples, to dyes commonly found in foods.

High performance liquid chromatography and gas chromatography use narrow tubes called columns packed with stationary phase, through which the mobile phase is forced. The sample is transported through the column by continuous addition of mobile phase. This process is called elution. The average rate at which an analyte moves through the column is determined by the time it spends in the mobile phase (Smith et al. 1965; Wagman and Cooper 1989; Carey 2003; Björnstad et al. 2009).

2.2.2.1 THIN LAYER CHROMATOGRAPHY (TLC)

Thin Layer Chromatography is a simple, quick, and inexpensive procedure that gives the chemist a quick idea as to the number of chemical components is in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_F of a compound is compared with the R_F of a known compound (Smith et al. 1965; Zullich et al. 1975; Kovac-Besovic and Duric 2003).

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase which slowly rises up the TLC plate by capillary action. As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried further up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are coloured, visualization is straightforward. Usually the compounds are not coloured, so a UV lamp is used to visualize the plates. The plate itself may contain a fluor which fluoresces everywhere except where an organic compound is on the plate (Smith et al. 1965; Zullich et al. 1975).

2.2.2.2 COLUMN CHROMATOGRAPHY (CC)

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical column (usually glass) and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture (Brimer and Dalgaard 1984; Eskew et al. 1984; Zhou et al. 2009).

The mixture to be analyzed by column chromatography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column. Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called flash chromatography (Eskew et al. 1984; Zhou et al. 2009).

If the compounds separated in a column chromatography procedure are coloured, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column chromatography are colourless. In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by thin layer chromatography. Other methods of analysis are available but most commonly thin layer chromatography is used in organic chemistry (Eskew et al. 1984; Zhou et al. 2009).

2.2.2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a form of column chromatography used frequently in analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds

chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used (Pavia et al. 1995; Daley and Daley 1996; Carey 2003).

OPERATIONS OF HPLC

The sample to be analyzed is introduced in small volume to the stream of mobile phase. The analytes motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time; the retention time under particular conditions is considered a reasonably unique identifying characteristic of a given analyte. The use of smaller particle size column packing to create higher backpressure increases the linear velocity giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combination of water or various organic liquids. The most common are methanol and acetonitrile. Water may contain buffers or salts to assist in the separation of the analyte components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent (Pavia et al. 1995; Daley and Daley 1996).

A further refinement to HPLC has been to vary the mobile phase composition during the analysis; this is known as gradient elution. A normal gradient for reversed phase chromatography under conditions of might start at 5% methanol and progress linearly to 50% methanol over 25 minutes; the gradient chosen depends on how hydrophobic the analyte is. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase composition relative to the stationary phase. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/methanol gradient, the more

hydrophobic components will elute (come off the column) when the mobile phase consists mostly of methanol (giving a relatively hydrophobic mobile phase). The more hydrophilic compounds will elute relatively low methanol/high water. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Often a series of tests are performed on the analyte and a number of trial runs may be processed in order to find the HPLC method which gives the best separation of peaks.

HPLC can either be analytical or preparative. In order to identify and quantify the component parts of a mixture Analytical Chromatography is employed. Preparative Chromatography as opposed to analytical chromatography is used to isolate specific quantities of a particular substance contained in a mixture. The basic difference between the two techniques is that the mixture is not merely monitored or analyzed but the individual solutes of interest are actually isolated, collected and recovered for further use (Neue 1997; Snyder et al. 2009).

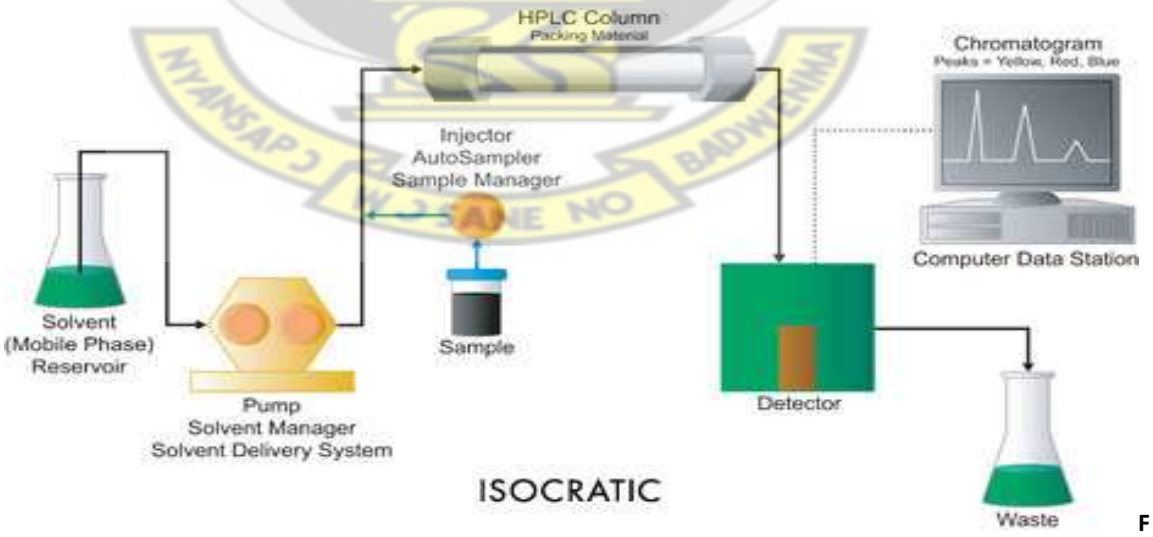


Figure 2-4: Typical set up of high pressure liquid chromatography

TYPES OF HPLC

PARTITION CHROMATOGRAPHY

Partition chromatography uses a retained solvent, on the surface or within the grains or fibres of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional coulombic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent.

Polar analytes diffuse into a stationary water layer associated with the polar stationary phase and are thus retained. Retention strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends on the functional groups in the analyte molecule which promote partitioning but can also include coulombic (electrostatic) interaction and hydrogen donor capability. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times (Neue 1997; Snyder et al. 2009).

NORMAL PHASE CHROMATOGRAPHY

Also known as Normal phase HPLC (NP-HPLC), or adsorption chromatography, this method separates analytes based on adsorption to a stationary surface chemistry and by polarity. It was one of the first kinds of HPLC that chemists developed. NP-HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased

analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time (Neue 1997; Snyder et al. 2009).

DISPLACEMENT CHROMATOGRAPHY

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities (Neue 1997; Snyder et al. 2009).

REVERSE PHASE CHROMATOGRAPHY (RPC)

A chromatogram of complex mixture (perfume water) obtained by reversed phase HPLC. Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water. Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligand's alkyl chains and can have problems entering the pores of the stationary phase.

Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the

overall surface area is decreased. Similarly organic compounds with single C-C-bonds elute later than those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond (Neue 1997; Snyder et al. 2009).

SIZE EXCLUSION CHROMATOGRAPHY

Size exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles on the basis of size. It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins. This technique is widely used for the molecular weight determination of polysaccharides. SEC is the official technique (suggested by European pharmacopeia) for the molecular weight comparison of different commercially available low-molecular weight heparins (Neue 1997; Snyder et al. 2009).

ION EXCHANGE CHROMATOGRAPHY

In ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. In general, ion exchangers favour the binding of ions of higher charge and smaller radius. An increase in counter ion (with respect to the functional groups in resins) concentration reduces the retention time. An increase in pH reduces the retention time in cation exchange while a decrease in pH reduces the retention time in anion exchange (Neue 1997; Snyder et al. 2009).

BIO-AFFINITY CHROMATOGRAPHY

This chromatographic process relies on the property of biologically active substances to form stable, specific, and reversible complexes. The formation of these complexes

involves the participation of common molecular forces such as the Van der Waals interaction, electrostatic interaction, dipole-dipole interaction, hydrophobic interaction, and the hydrogen bond. An efficient, biospecific bond is formed by a simultaneous and concerted action of several of these forces in the complementary binding sites (Neue 1997; Snyder et al. 2009).

AQUEOUS NORMAL PHASE CHROMATOGRAPHY

Aqueous normal phase chromatography (ANP) is a chromatographic technique which encompasses the mobile phase region between reversed-phase chromatography (RP) and organic normal phase chromatography (ONP). This technique is used to achieve unique selectivity for hydrophilic compounds, showing normal phase elution using reverse-phase solvents (Neue 1997; Snyder et al. 2009).

2.3 INSTRUMENTAL METHODS OF ANALYSIS

A known botanical compound can usually be identified by its spectral characteristics. These are ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) measurement. The spectral characteristics are compared with authentic material or with data from literature for confirmation of the identity of the compound. The data is sufficient to characterize the structure, however chemical degradation or preparing the compound by laboratory synthesis is used to confirm the identity. X-ray crystallography is also used to identify substances obtained in crystalline form (Carey, 2003).

2.3.1 INFRARED (IR) SPECTROMETRY

Infrared (IR) spectroscopy is the measurement of absorption of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification (Carey, 2003).

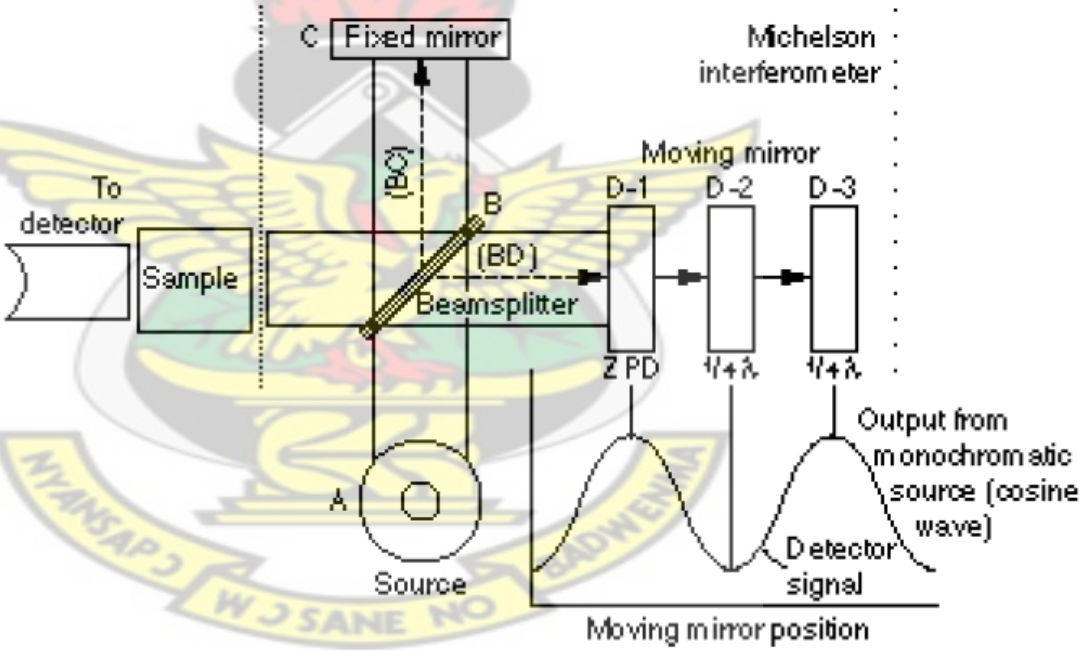


Figure 2-5: A simplified optical layout of a typical FTIR spectrometer.

There are three basic spectrometer components in an FT system: radiation source, interferometer, and detector. The interferometer, divides radiant beams, generates an optical path difference between the beams, and then recombines them in order to

produce repetitive interference signals measured as a function of optical path difference by a detector. As its name implies, the interferometer produces interference signals, which contain infrared spectral information generated after passing through a sample and is eventually focused on the detector Pavia et al. 1995; Daley and Daley 1996).

2.3.2 MASS SPECTROMETRY

Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. It is also used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds (Pavia et al. 1995; Daley and Daley 1996).

The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios. MS instruments consist of three modules: an *ion source*, which can convert gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase); a *mass analyzer*, which sorts the ions by their masses by applying electromagnetic fields; and a *detector*, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds (Pavia et al. 1995; Daley and Daley 1996).

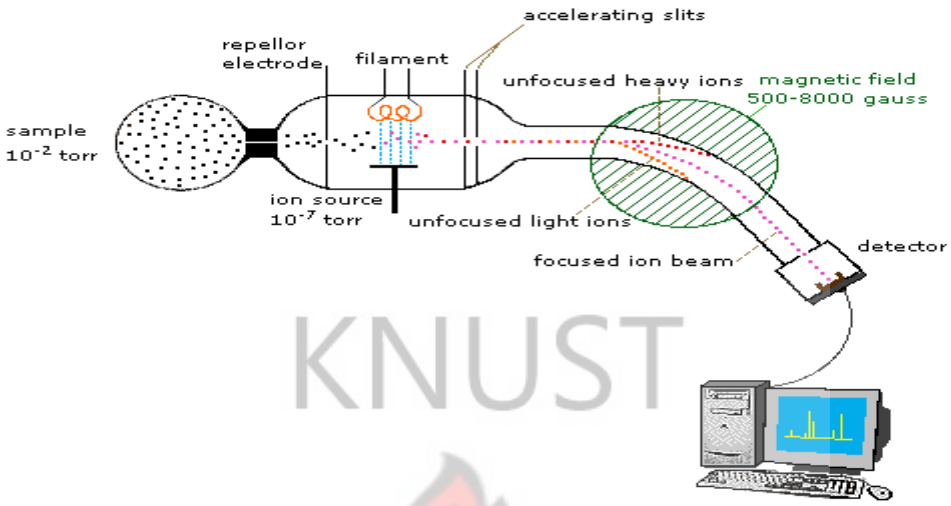


Figure 2-6: Schematic layout of mass spectrometer

2.3.3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

This spectroscopic technique is used primarily to elucidate the structures of organic compounds, especially following synthesis or isolation of products. It is based on the fact that atomic nuclei that have an angular momentum and a magnetic moment have a special property called nuclear spin. For example, protons (^{1}H) and carbon-13 (^{13}C) have nuclear spin while oxygen (^{16}O) and carbon-12(^{12}C) do not. The ^{1}H and ^{13}C nuclei, which due to their inherent spin are most commonly examined in NMR experiments, act like small magnets in a magnetic field as they align themselves parallel with or anti-parallel to the applied magnetic field. The parallel orientation is lower in energy and therefore preferred (Pavia et al. 1995; Daley and Daley 1996).

Nuclear magnetic resonance exploits this interaction of spin with strong magnetic fields by using radiofrequency (RF) radiation to stimulate transitions between different nuclear spin states of sample in a magnetic field. When irradiated, the parallel, lower energy nuclei move to the higher energy spin state where they are now in resonance.

This is akin to electrons being in the 'excited state' in UV spectroscopy. When the pulse of irradiation disappears, the nuclei relax to the lower energy spin state once more. This data is subjected to Fourier Transformation (FT) to yield a spectrum giving information on each type of nucleus in the molecule. However, because the nuclei are surrounded by other electrons and atoms giving it shielding effect, more than one signal may be observed for each type of nucleus. Three important spectral parameters are obtained in a ^{1}H-HMR spectrum: chemical shifts, coupling constants and intensities (integrals). These provide information on the environment and proximity of the structure groups, the molecular structure and the nuclei involved respectively (Pavia et al 1995 Carey 2003).

The nuclides of most interest are protons (^{1}H) and carbon-13 (^{13}C) for organic molecules, though others such as phosphorous and silicon can be used. NMR spectroscopy is most useful as a qualitative tool for determining the structure and identity of molecules. It is rich in information content but can be poor in sensitivity. Most NMR instruments today are based on FT-NMR (Carey 2003).

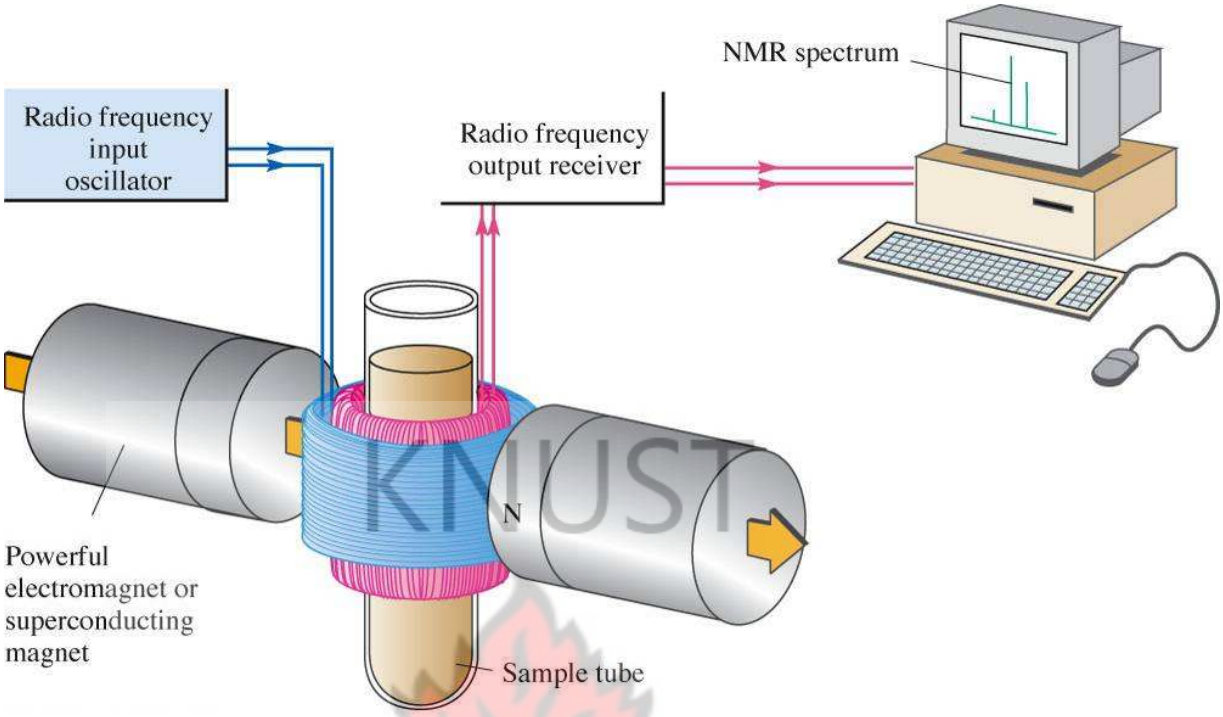


Figure 2-7: Schematic diagram of a nuclear magnetic resonance spectrometer

SOURCE

Radiofrequency (RF) transmitters generate frequencies of a few MHz to almost 1 GHz, which irradiate the sample molecules. If the energy difference between the relevant spin states is matched by the RF pulse, the nuclei will move to the higher spin state and be 'in resonance' with the magnetic field (Carey 2003).

DETECTOR

When the resonant condition is met, the NMR signal is collected at the RF receivers. NMR signals are generally weak and need to be amplified and processed prior to further analysis. Using the pulsed mode, the free induction decay (FID) spectrum in the time domain is recorded and while it contains all the information on frequencies, splitting and

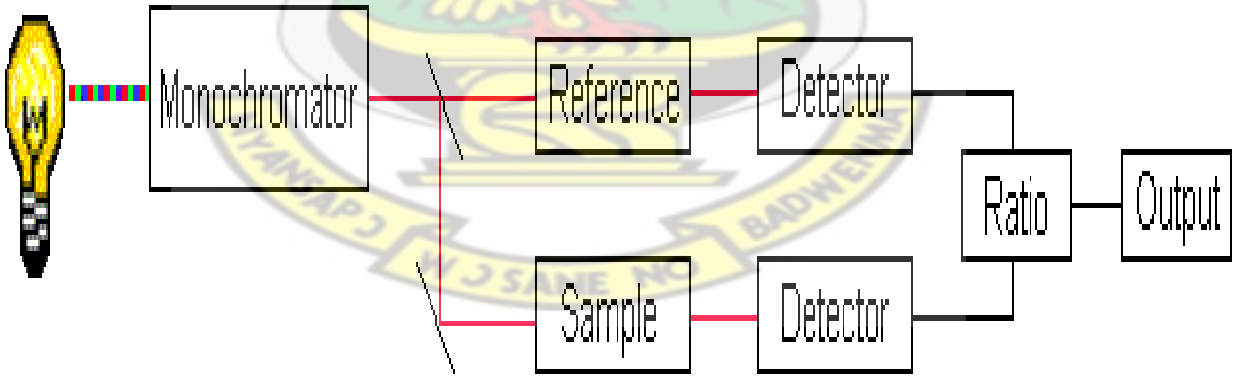
integrals, it must be converted into the frequency domain by Fourier Transformation (FT) (Carey 2003).

OUTPUT

At the computer, the huge amount of information is processed and spectral searching and matching can be carried out. NMR spectra can be very complex, especially two-dimensional (2-D) experiments, and may require detailed data analysis and interpretation (Carey 2003).

2.3.4 ULTRAVIOLET AND VISIBLE (UV-V) SPECTROSCOPY

It is used to measure the absorption of U.V. or visible radiation. Figure 3 shows a schematic diagram of a double-beam UV-Visible spectrophotometer. The parts consist of a light source UV and visible, wavelength selector (monochromator), sample and reference containers, detector, signal processor and readout (Carey 2003).



A schematic diagram of ultraviolet and visible (UV-V) spectroscopy

2.3.5 POLARIMETRY

A sample that contains only one enantiomer of a chiral molecule is said to be optically pure. The enantiomer that rotates light to the right, or clockwise when viewing in the direction of light propagation, is called the dextrorotatory (d) or (+) enantiomer, and the enantiomer that rotates light to the left, or counter clockwise, is called the levorotatory (l) or (-) enantiomer (Daley and Daley 1996, Carey 2003).

Optical rotation occurs because optically active samples have different refractive indices for left- and right-circularly polarized light or left- and right-circularly polarized light travel through an optically active sample at different velocities. This condition occurs because a chiral centre has a specific geometric arrangement of four different substituents, each of which has a different electronic polarizability. Light travels through matter by interacting with the electron clouds that are present. Left-circularly polarized light therefore interacts with an anisotropic medium differently than does right-circularly polarized light.

Linearly or plane-polarized light is the superposition of equal intensities of left- and right-circularly polarized light. As plane-polarized light travels through an optically active sample, the left- and right-circularly polarized components travel at different velocities. This difference in velocities creates a phase shift between the two circularly polarized components when they exit the sample. Summing the two components still produces linearly polarized light, but at a different orientation from the light entering the sample (Daley and Daley 1996, Carey 2003).

INSTRUMENTATION

The simplest polarimeter consists of a monochromatic light source, a polarizer, a sample cell, a second polarizer, which is called the analyzer, and a light detector. The analyzer is oriented 90^{o} to the polarizer so that no light reaches the detector (Carey 2003).

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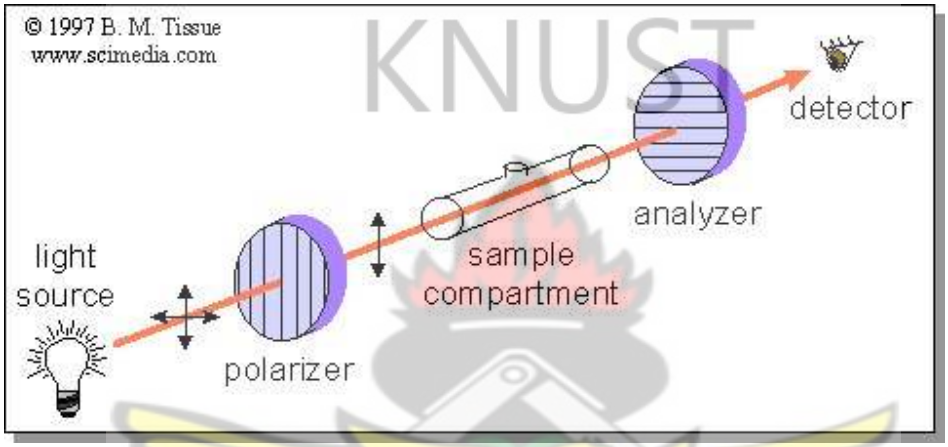

Figure 2-8: A schematic of a polarimeter

When an optically active substance is present in the beam, it rotates the polarization of the light reaching the analyzer so that there is a component that reaches the detector. The angle that the analyzer must be rotated to return to the minimum detector signal is the optical rotation, α.

The amount of optical rotation depends on the number of optically active species through which the light passes, and thus depends on both the sample path length and the analyte concentration. Specific rotation, $[\alpha]$, provides a normalize quantity to correct for this dependence, and is defined as:

$$[\alpha] = \frac{\alpha}{l \cdot d}$$

where $\alpha$is the measured optical rotation in degrees, l is the sample path length in decimetres (dm), and d is the density if the sample is pure liquid, or the concentration if the sample is a solution. In either case, the units of d are g/cm^3.

The specific rotation of a chemical compound [α] is defined as the observed angle of optical rotation α when plane-polarized light is passed through a sample with a path length of 1 decimetre and a sample concentration of 1 gram per 1 millilitre. The specific rotation of a pure material is an intrinsic property of that material at a given wavelength and temperature. Values should always be accompanied by the temperature at which the measurement was performed and the solvent in which the material was dissolved. Often the temperature is not specified; in these cases it is assumed to be room temperature. The formal unit for specific rotation values is deg dm^{-1}cm^3 / g but scientific literature uses just degrees. A negative value means levorotatory rotation and a positive value means dextrorotatory rotation (Carey 2003).

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2.4 PLANTS WITH ANTITERMITIC ACTIVITIES

Botanical and anthropological studies have shown that some forest plants or material extracted from them have both insect- and microbial-resistant properties and are useful for preventing and controlling insect pests (Apantaku 1999; Cobbinah et al. 1999). For example, the jatropha seed, leaf, and root extracts have been found to provide protection against insect pests and bacterial diseases of plants in the Amazon (Verma et al. 2009). In West Africa, the leaves, fruits and seeds of neem are used to make natural pesticides that keep insects away from vegetables and stored grains (Apantaku 1999). Table 1 shows some of forest plants used traditionally in West Africa for pest control. Those of interest to this study are described further below.

Table 2-8: Some forest plants used traditionally in West Africa for pest control (Apantaku 1999).

Botanical Name	Part Used	Use
Azadirachta indica A.Juss.	Fresh leaf	Control of cowpea weevils, maize weevils and leafhopper, thrips, and red mites on citrus.
Citrus medica L.	Fresh fruit juice	Termite and flies control on farm.
Elaeis guineensis A.Chev.	Palm frond and palm oil	Control of rats on stored yam.
Cocos nucifera L.	Coconut oil	Controls insects on Cochorus olitorus.
Baphia nitida Lodd.	Fresh roots	Prevents birds (weaver) attack on rice.
Citrus medica L	Fresh fruit juice	Multi-purpose insecticide for melon cowpea, and leafy vegetables.
Treculia africana Decne. ex Trécul	Fresh leaf	Control of insect pests on yam tubers and cassava cuttings before sprouting.
Ficus capensis Thunb.	Fresh or dried fruits	Instecticide/pesticide for planted melon seeds.
Theobroma cacao L.	Ash from pods burnt dried	Prevention of foliage pests on leafy and fruit vegetables.
Englerina gabonensis (Engl.) Balle	Leaf	Insecticide for melon (against moth, weevils, beetles, and grasshopper).
Alchornea cordifolia (Schumach.) Müll.Arg.	Fresh fruit	Multi-purpose insecticide on melon.
Nicotiana tabacum L.	Live plant/leaf	Snake and insect repellant from farm.
Citrus medica L	Fresh fruit juice	Insect repellant for stored rice and maize produce.
Jatropha gossypifolia L.	Live plant	Termite repellant from the farm.
Capsicum frutescens L.	Fresh or dried stem	Yam and casava pests and insects prevention and repellant.
Psorospermum corymbiferum Hochr.	Fresh or dried root	Prevents partridge attack on planted seeds.
Hoslundia opposita Vahl	Live plant	Snakes and chewing insects repellant from the farm.
Capsicum annuum L.	Dried ripe fruit	Used torepel prevent monkeys from the farm.
Ocimum gratissimum L.	Live plant	Repels rats and chewing insects.
Hyptis spicigera Lam.	Fresh/dried plant	Protect against leaf chewing insects.
Pouzolzia guineensis Benth.	Fresh/dried leaf	Insecticide/pesticide on cassava.
Momordica charantia L.	Fresh leaf	Insecticide on planted yam-sets.
Allium sativum L.	Live plant/cloves	Insecticide and pests repellant.

CASSIA NIGRICANS

This is a woody annual herb or shrub which grows up to 1.2 – 1.5 m high and bears small yellow flowers (Figure 2-10). It is widespread in India, Arabia, Northern Nigeria and other tropical African countries, especially in cultivated ground or old clearings by roadsides and open grassy areas (Akah et al. 1998; Belmain et al. 2001; Georges et al. 2008)

CYMBOPOGON GIGANTEUS

This is a loosely tufted perennial grass with erect culms, sometimes stilt-rooted, to about 2½ m high (Fig 2-9). It occurs in deciduous savanna bushland and wooded grassland and abundant throughout the region and in general over all of tropical Africa, with var. Inerm restricted solely to Mauritania and Mali. This grass is dominant over large regions of the savanna constituting the major part of the herbaceous flora. It requires good soil and no shade, often colonising fallows and fire-devastated areas. It prevents soil erosion and has both prophylactic and curative power against fever, yellow fever and jaundice (Alitonou et al. 2006; Boti et al. 2006; Nyamador et al. 2010).

Figure 2-9: ***Cymbopogon giganteus***

Figure 2-10: ***Cassia nigricans***

Figure 2-11: ***Jatropha curcas***

Figure 2-12: ***Carapa procera***

Figure 2-2-1: ***Vetiver zizaniodes***

Figure 2-1-2: ***Thevetia peruviana***

Figure 2-1-3: ***Hyptis spicygera***

Figure 2-1-4: ***Chromolaena Odorata***

CARAPA PROCERA

Carapa procera DC belongs to the family of plants called Meliaceae. In Ghana it is commonly referred to as Bete or Krupi. It is widely distributed in western parts of tropical Africa and extending eastward to Uganda as well as in the Amazon of tropical America. Its habitat range includes lake-shores, riparian and mid-altitude forest, especially where drainage is impeded, and typically at 1100-1800 m altitude. It has also frequently been recorded growing on sandy soils, generally at sea level. It is reported to be highly medicinal (Oliver-Bever 1986). The wood is reported to be resistant to termites (Mikolajczak et al. 1988; Konan et al. 2003; Forget and Jansen 2007).

CHROMOLAENA ODORATA

Chromolaena odorata R. M. King and H. Robinson (formerly *Eupatorium odoratum*), generally known as the Siam weed, is a perennial scrambling shrub native to the neotropics (Owusu 2000). It is a member of the family of plants known as Asteraceae. It is a fast-growing perennial and invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevents the establishment of other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic properties and growth inhibitors. The plant can be poisonous to livestock as it has exceptionally high level of nitrate (5 to 6 times above the toxic level) in the leaves and young shoots; the cattle feeding on these die of tissue anoxia (Steenkamp et al. 2004; Antwi-Boasiako and Damoah 2010; Srinivasa Rao et al. 2010; Van Driesche et al. 2010).

In Ghana, it is popular known as “Acheampong weed”. The present distribution of *C. odorata* in Ghana is as far north as 8^{o} and 15' latitude. In Ghana the herbs are cut and sandwiched between maize layers during the process of packing on a barn. It is also used to control bed bugs in some localities. The leaves are normally used. Farmers believe that odour from the plant has a potential of driving away insects. Apart from that it is also used to dress wounds and also as a preservative for cadavers up to about two days (Irobi 1992; Baruah and Leclercq 1993; Phan et al. 1998).

HYPTIS SPICIGERA

Hyptis spicigera Lam. is an erect hairy aromatic herb commonly found in the bushlands of southern Sudan, and western Kenya. It is used as a trap plant against *Striga hermonthica* weed as well as an insect repellant in grain stores (Fragoso-Serrano et al. 1999). It is also used as a remedy for stomach ache and as a source of flavouring for pharmaceuticals (Kouninki et al. 2005; Bum et al. 2009).

VETIVER ZIZANIODES

Vetiver grass, *Vetiveria zizanioides* Nash is a native plant of Indian whose domesticated type is cultivated worldwide in tropical and subtropical regions for its efficacy in the measurement of soil erosion and for the commercial importance of its oil, extracted from the roots. Many soaps, perfumes and after-shaves include vetiver oil as active ingredient. Moreover, nootkatone, one of the 300 components of vetiver oil, is used to aromatize drinks with its distinctive grapefruit flavour (Maistrello et al. 2001; Zhu et al. 2001b; Maistrello et al. 2003).

Chen (2004) observed that the vetiver plant grown in close proximity to sugar cane could inhibit to a very substantial degree the attack upon the sugar cane of insects such as the cane borer (Chen et al. 2004). Likewise, a farmer in Louisiana reported that in a plot of crop where vetiver was used as mulch, no insects of any kind ever came near. It has also been found that the tops of vetiver, in the same formation of mixture with the residue of the roots, will make an absolute repellent for the insects that may damage strawberries grown in southern U.S. Recently, Maistrello and Henderson found a group of compounds, such as nootkatone, in vetiver roots, which were able to disrupt termite behaviour and physiology as a consequence of direct physical contact, ingestion, or exposure to the vapours (Maistrello et al. 2001; Maistrello et al. 2003). They also found that ingestion of wood treated with vetiver oil or nootkatone causes the progressive death of the protozoa living inside the termite gut, ultimately resulting in a progressive decline of its colony through starvation, as these termites rely on the protozoa for the digestion of their wooden food.

JATROPHA CURCAS L (PHYSIC NUT)

Jatropha is native to Central America and has become naturalized in many tropical and subtropical areas, including India, Africa and North America. Originating in the Caribbean, *Jatropha* was spread as a valuable hedge plant to Africa and Asia by Portuguese traders. Jatropha is a perennial shrub and the mature small trees bear separate male and female flowers.The shrub which does not grow very tall (normally up to 5 m high), belong to the family Euphorbiaceae or spurge family, *Jatropha* contains compounds that are highly toxic.

The hardy *Jatropha* is resistant to drought and pests, and produces seeds containing 27-40% oil wih an average of about 34.4%. The remaining press cake of jatropha seeds after oil extraction could also be considered for energy production. The fruit and the seed are reported to contain a contraceptive principle. The seed has insecticidal properties (Abdul Rahuman et al. 2008; Phowichit et al. 2008).

THEVETIA PERUVIANA

Thevetia peruviana is an evergreen flowering shrub belonging to the Dogbane family, Apocynaceae. In Ghana it is popularly known as milk bush. It grows in both temperate and tropical climate throughout the world. Its generic name is yellow oleander. It is an ornamental shrub, which grow to about 10 to 15 feet high. The leaves are spirally arranged, linear and about 13 to 15 cm in length. The flowers are bright yellow and funnel-shaped with 5 petals spirally twisted. The fruits are somewhat globular, slightly fleshly and have a diameter of 4 to 5 cm. The fruits which are green in colour become black on ripening. Each fruit contains a nut which is longitudinally and transversely divided. Its leaves are long, lance shaped and green in colour. The leaves are covered in waxy coating to reduce water loss. Its stem is green turning silver/gray as it ages. Thevetia peruviana has been extensively explored for its nutritional and medicinal values by various researchers. However, data on its pesticidal effects is sparse.

All parts of the plant contain a milky juice that is poisonous to man, animals and certain insects. This is due to the presence of at least 8 cardiac glycosides which the body selectively concentrates in the heart muscle. The seed kernels contain the highest concentrations of toxins. Thevetin A and B are found in the seed kernels, leaves and the

bark of the roots and stems. Thevetin is a bitter glycoside with potent cardiac action similar to that of digitalis, with 1/8 the strength of Ouabain. The leaves have been reported to contain iridoid glycosides, flavonoids, triterpenes, monoterpenes and cardiac glycosides. The seeds of Thevetia have been known to have insecticidal properties (Abe et al. 1995; Gata-Gonçalves et al. 2003; Bandara et al. 2010).

The tree is most commonly cultivated for its attractive yellow flowers. The lightweight, hard, gray wood is easily worked and has a fine texture. A bright-yellow, non toxic oil suitable for food or soap making can be extracted from the seeds. The oil (non-toxic when pure) is composed primarily of oleic, linoleic, stearic and palmitic acids. *Thevetia peruviana* is a source of oil for industry in China. The folk medicinal use of the seed oil in treating burns and infected wounds has been supported by the discovery that one of the fractions distilled from the seed oil is active against common infective bacteria Staphylococcus aureus, Streptococcus pyrogenes, Escherichia coli and Pseudomonas aeniginosa. The flesh of the fruits covering the seed is reported to be edible. The seeds are used as beads on necklaces and carried as pocket charms. The pulp of the fruits is reported to be eaten with impunity by chickens, livestock and humans, but this would seem imprudent. Thevetin the glycoside from T*hevetia peruviana* has been used medically to treat mild myocardial insufficiency in the presence of digitalis intolerance. In Russia, it is used for cardiac insufficiency with shortness of breath, and for ventricular insufficiency due to high pressure and atherosclerosis.

T.peruviana's seeds, leaves, fruits and roots has been used in traditional medicine as a purgative, as an emetic and for intermittent fever treatment. The extract is used in folk

medicines and there are reports that long-term use of oleander may have positive effects in patients with prostrate or breast cancer (Samal et al. 1992; Basile et al. 1993); it can grow in degraded soil and harsh weather condition and is thereby good for reclaiming degraded soil, oleander has been used as an abortifacient, to treat congestive heart failure, malaria, leprosy, indigestion, ringworm, venereal disease and even as a suicide instrument.

With partial hydrolysis and the loss of two glucose units, Thevetin A yields the therapeutic cardioactive drug peruvoside. One research report states that when Thevetin B is stripped of its sugar component, it is identical to digitoxin (a clinical useful cardiac glycoside). The presence of the anticancer compounds cerberin and ursollic acid may be the basis for the use of leaf poultices to treat tumours in Latin America. Folk medicine has used the sap to treat aching teeth, chronic sores, ulcers and mange. The bark, leaves, roots and seeds although often recognized as toxic, have been used in various formulations to treat bladder stones, oedema, fevers, insomnia, haemorrhoids, malaria and snakebite and to intoxicate fish for capture. Juice extracted from the leaves has been mixed with meat bait and used to kill nuisance tigers near Malay villages. Aucubine, an iridois heteroside extract from the leaves and fruit is an effective insecticide (Abe et al. 1995).

CHAPTER THREE

3. MATERIALS AND METHODS

The study consists of series of experiments carried out to identify the most efficacious plant material and characterise the active compound/s in the most efficacious extract that could be used to control termites. This chapter describes in detail the materials, processes and experiments including identification and collection of plant and termite samples; efficacy testing; extraction by three organic solvents, analysis of the most efficacious extract by thin layer chromatography, separation of the extract by column and high pressure liquid chromatography methods and analysing the component by spectrometry methods including mass spectrometry, nuclear magnetic resonance and infra red to identify the active ingredients.

3.1 MATERIALS

This section indicates the lists of chemicals, reagents, glass wares, equipment, plant samples, test organisms and wood samples employed in conducting the various experiments (Table 3-1). Chemicals and reagents were obtained from Scharlau Chemical Limited, Poole, England or British Drug House, Poole England. They were of analytical grade unless otherwise stated.

Table 3-1: Materials employed in conducting various experiments

EQUIPMENT AND GLASSWARES	CHEMICALS AND REAGENTS	PLANT MATERIALS	TEST ORGANISMS	WOOD SAMPLES
Soxhlet Extractor	Distilled Water	*Thevetia peruviana* (pers) K Shum powder (root, leaves seed)	*Microtermes species*	*Triplochiton scleroxylon*
Rotary Evaporator	Sodium Picrate	*Cassia nigricans* Vahl powder	*Macrotermes bellicosus* (Smeathman),	
Funnels	H_2SO_4	*Cymbopogon ginganteus* (Hachst) Chiov leaves powder	*Pseudocanthotermes militaris* (Hagen)	
Electrical Oven	Butanol	*Carapa procera* DC leaves powder	Brine shrimps	
Petri Dishes	NaOH	*Chromolaena odorata* (L.) R. M. King and H. Robinson, powder (leaves and stem)		
Refrigerator	Benzene	*Hyptis spicigera* Lam powder		
Micro Pipettes	Fehlings Solution	*Vetiver zizaniodes Nash* powder *(leaves and roots)*		
Electric Mill	Propanol	*Thevetia peruviana* root pet ether extract		
Beakers	HCl	*Thevetia peruviana* root ethanol extract		
Mettler balance	Methanol (HPLC grade)	*Thevetia peruviana* root water extract		
Measuring Cylinders	Ethanol	*Jatropha curcas* L seed powder		
Pipettes	KBr	*Jatropha curcas* L root powder		
Beakers (10ml, 100ml, 500ml, 1000ml)	NH_3			
Conical flasks	KOH			
Sample bottles	Chloroform			
Hoods	Acetic Anhydride			
Thermo hydrometers	Antimony Trichloride			
Chromatography columns	Petroleum Ether			
Desiccators	Silica gel			
Erlenmeyer flasks	Acetonitrile (HPLC grade)			
TLC Tanks and plates	Dursban			
Water Bath				
Automatic Shaker				
Separatory funnel				
Volumetric flasks				

3.2 PROCEDURE OF THE EXPERIMENTS

The procedures followed during experiments carried out are shown in Figure 3-1 below.

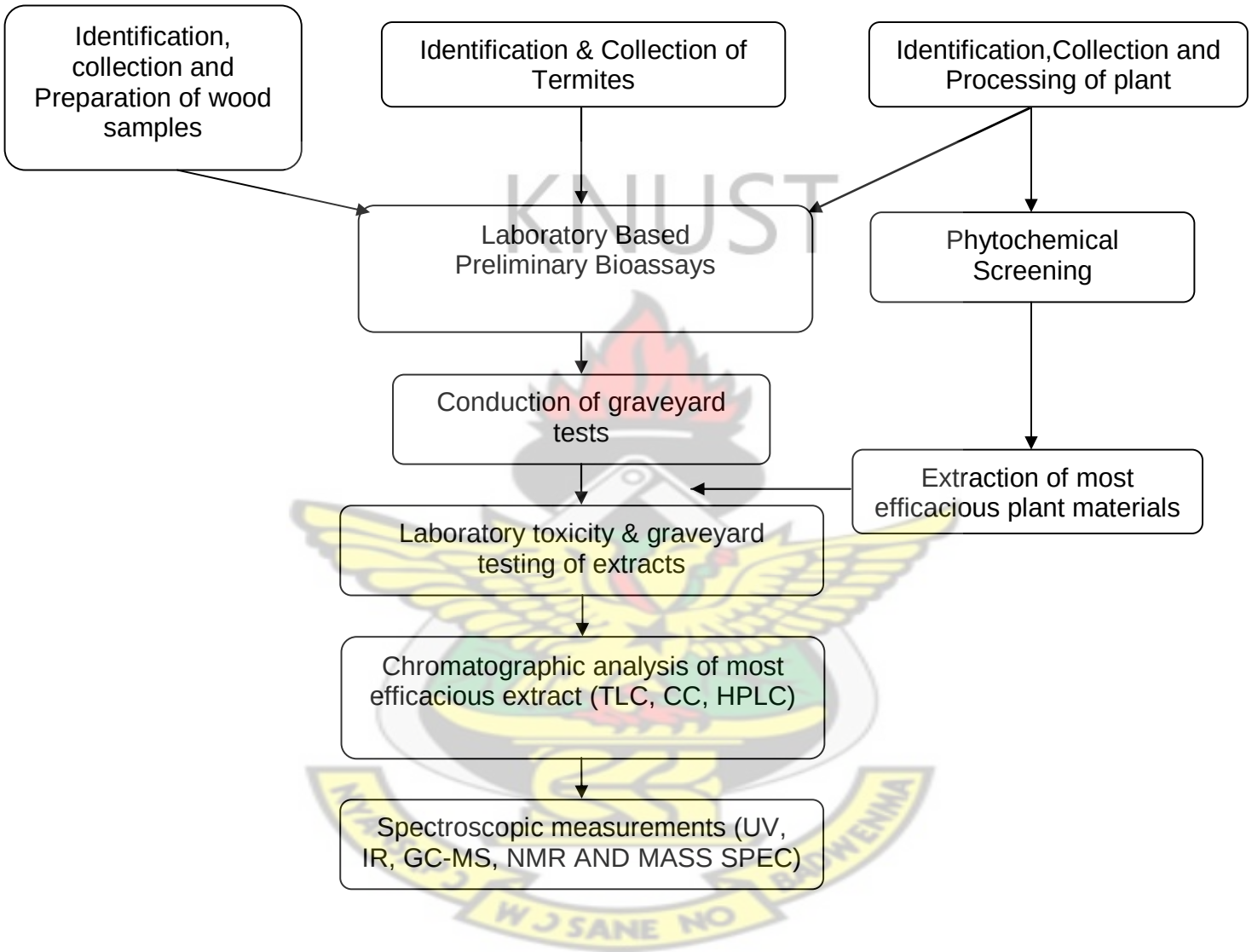


Figure 3-1: A schematic diagram showing the stepwise procedures adopted in the conduct of the experiments.

3.2.1 COLLECTION, IDENTIFICATION AND PREPARATION OF PLANT AND TERMITE SAMPLES

This stage involved collection, identification and preparation of plant samples, termite species wood samples and termite testing site.

3.2.1.1 PLANT SAMPLES

Eight different test plants were collected from various parts of Ghana for identification prior to setting up the experiments. The plants are *Thevetia peruviana* (Pers) K Shum (yellow oleander); *Carapa procera* DC (Monkey Kola), and *Jatropha curcas* L all of which were obtained from Kwame Nkrumah University of Science and Technology, Kumasi. *Cassia nigricans* Vahl, C*ymbopogon ginganteus* (Hachst) Chiov (Tsauri grass), *Hyptis spicigera* Lam., (American bushmint were obtained from Navrongo and Bolgatanga , V*etiver zizanioides* Nash (Vetiver grass) was collected from the Building and Road Research Institute Fumesua near Kumasi and *Chromolaena Odorata* (L.) R. M. King and H. Robinson also known as "Siam weed" from Pakyi No. 2 near Kumasi. Collections were made in between March and July 2005. The plants were kindly identified at the herbarium, Department of Botany, University of Ghana, Legon (courtesy Mr. John Amponsah). These plants were selected because they have been reported to be used locally in Ghana to protect stored cereals and pulses against pests (Cobbinah et al. 1999).

The plants were separated into different parts (roots, stems, flowers and seeds) and air dried. The dried parts were ground separately by plant using the Thomas electric mill to a uniform texture (Sieve aperture size 3mm), and the 40-60 mesh particles were collected.

Ground samples were sealed in air-tight bags. About 1000 grams of samples were prepared for further extraction. The ground products were collected in clean dry polythene bags separately, labelled and stored in the refrigerator at 4^{o} C until used.

3.2.1.2 WOOD SAMPLES

Test blocks of wood were cut from the sapwood of *Triplochiton scleroxylon*, K. Schum, (Obeche) that was obtained from a Sawmill in Ahensan Kumasi. This bait wood is well known to be very susceptible to termite attack, the sapwood being more susceptible than the heartwood (Ocloo 1973). Test blocks measuring 10cm × 4.1cm × 2.6 cm were prepared and were conditioned by oven-drying for one week at 30^{o}C and weighed periodically until constant weights were attained. These test blocks were used for the field test.

3.2.1.3 TERMITE CULTURE

Termites were collected from the field and identified to the species level using keys and literature provided in (Wagner et al. 1991). Identifications were confirmed by the kind courtesy of Dr John Ocloo, formerly of CSIR-BRRI, Ghana. They were collected, cultured in metal cans and maintained in laboratory conditions at room temperature of 25-30^{0}C and 70-80% RH. The termites were fed with pieces of moistened wood (obeche). These were used for the laboratory bioassay. At the time of assay the termites had been held in the laboratory for up to 14 days.

3.2.1.4 TERMITE TESTING SITE

The field tests were carried out at the BRRI termite testing site. The test site was an old termite testing site of the Materials Research Division of the CSIR-BRRI at Fumesua. This is a forest site near the village of Fumesua (6^{o}42' N, 1^{o}31' W), situated about 12 km east of the city of Kumasi.

The ecology of the termite species on this test site has been described by (Usher and Ocloo 1975), and a list of the species attacking timber is given in Table 3-2. Prior to the use of the site the area was a farm scrub, having been cultivated and abandoned for about one year. The scrub re-growth was then cut down leaving scattered trees and bushes so that access to the whole site was easy and that a large portion of the site was shaded from the sun for most of the day. By the time that the experiments were begun there was an almost complete ground cover of grasses, and during the experiments there was little visual change in the test site.

Table 3-2: Termite species found either damaging or in contact with the wood samples in the graveyard tests at Fumesua – a forest test site by (Usher and Ocloo 1975).

Termites: family and species	**Number of timber species on which this termite was found**
Rhinotermitidae (Coptotermitinae)	
Coptotermes irttermedius Silvestri	13
Termitidae (Amitermitinae)	
Amrtermes evuncifer Silvestri	26
Termitidae IMacrotermitinae)	
Anctstrotermes spp. (mostly *A. crutifer* (Sjostedtl) but with the occasional *A. guineensis* (Silvestri))	59
Macrotermes spp. (both *M. Betlicoms* (Srneathman) and *M. subhyalinus* (Rambur) were present, but the latter was more frequent)	67
Microtermes subhyalinus Silvestri	16
Odontotermes pauperans (Silvestri)	7
Psaudaconthotermts militaris (Hagen)	44
Termitidae (Nasutitermitinae)	
Nasutitermes fatifrons (Sjostedl)	25

3.2.1.5 TERMITE SPECIES ON TEST FIELD

To demonstrate that there were termites in the test site, the following activities were carried out. An indirect method of sampling the termite species was adopted. This method consisted of inserting in the soil eight hundred (800) wood blocks, which would act as lures, and recording the termite species that were attracted to these bait wood blocks over a period of one year. The baiting program was done exclusively with sapwood of *Triplochiton scleroxylon* (obeche) which measured approximately 10cm along the grain, 4 cm tangentially and 2.6 cm radially. The blocks were buried in the ground with their tangential and radial dimensions vertical and approximately 1cm remaining above ground to facilitate location and extraction. The blocks were loosely covered by the surrounding litter of leaves, twigs and grass; and the location was marked with a vertical stick approximately 25cm from the block. The blocks were laid out on a grid, 2m by 1m of approximately 40 bait woods per each row. All blocks were inspected every month – 28 days. The blocks were located and quickly pulled out of the soil. Notes were made of the termites species present, of any artefact of recognizable damage. When the identity of the termite species was in doubt, a few species were collected and stored in 70% ethanol for subsequent identification. All the blocks that had been completely damaged by termites were replaced with new blocks, and undamaged blocks were re-inserted. In all twelve readings were taken over the 1 year test period.

3.2.2 BIOASSAY OF PULVERISED PLANT MATERIALS

A series of tests were carried out both in the feld and the laboratory to identify the plant material with most promising antitermitic properties.

3.2.2.1 PRELIMINARY BIOASSAY OF PULVERIZED PLANT MATERIALS

Laboratory assays were performed to detect the presence of natural termite controlling activities in various parts of five plants. These plants are *T. peruviana, C. nigricans, C. ginganteus, V. Zizanioides* and *H. spicigera*. The assay involved a test for survival of subterranean termites, when isolated and exposed to pulverized plant products (leaves, seeds, stem and roots) and monitored over a 2 day period.

Half a gram each of the powdered plant samples was weighed into 5cm Petri dishes and moistened with 1ml portions of distilled water. Twenty termites were counted with light feathers and added. The Petri dish was then covered with a mesh to allow for aeration and prevention of the termites from moving out or foreign materials from entering. Controls consisted of a 5cm filter paper moistened with 1ml portions of distilled water. Wooden hoods were constructed and covered with black polythene. Relative humidity in the hoods was maintained at by placing glycerine/water mixture in one corner of the hood. The petri dishes containing plant samples were placed in the hood. The numbers of dead termites were recorded every 2 hours over 48 hours. This enabled the calculation of total mortality (i.e the number of dead termites over a period of 48hrs) from exposure to various test materials This assay was limited to only one species of termites i.e. *Macrotermes bellicosus* so it was discontinued.

3.2.2.2 FIELD TEST OF GROUND PLANT MATERIALS

The field tests to assess the termite controlling properties of the plant materials were carried out by adopting a 'Graveyard Test' method. The method exposes the plant materials to termite species in their natural habitat (Edwin and Ashraf 2006; Antwi-Boasiako and Allotey 2010). The test site was an old termite testing site of the Materials Research Division of the Council for Scientific and Industrial Research-Building and Road Research Institute at Fumesua. The aims were to assess the antitermite capabilities of these pulverized plant materials on the field and to select the most efficacious among them. The 'Graveyard test' was repeated four times; each involving different sets of plants on different plots.

Experimental Design

The first test (experiment 1) involved the following plant materials, *T. peruviana, C. nigricans, C. giganteus, H. spicigera* and *V. zizaniodes (leaves and root)* and was conducted on seven test plots. Each plot of the test block measured approximately 1.20 × 0.40m. Test blocks of *Triplochiton scleroxylon,* 10cm × 4.1cm × 2.6cm were completely buried in a grid of 7 rows × 3 columns, at 20 cm apart.

At each position of test block, the soil was excavated to a depth of 10 cm which would enable the test block to be completely buried. About 450g (four hundred and fifty grams) of the excavated soil was mixed with 10g of the pulverized material of the requisite test plant i.e. *T. peruviana, C. nigricans, C. giganteus, H. spicigera and V. zizaniode (leaves and root).*The test stakes were placed in the excavated holes with its long axis vertical and the treated soil was evenly placed back to cover the block. Each test plant material was replicated three times on each plot.

Each graveyard test lasted 8 weeks and was inspected twice at 4 week intervals. At the first inspection termite damage was visually assessed and the damage rated accordingly by visually inspecting them. At the second and final inspection, the samples were harvested, assessed visually, washed in water, air dried for two days, oven dried at 50^{o}C and weighed till constant weights were attained.

The design described above was repeated for the following sets of pulverized plant materials;

1. *C. procera,* C*. odorata* (stems and leaves) and *T. peruviana* (roots) (experiment 2).
2. *T. peruviana* and *J. curcas* (roots and seeds) (experiment 3).
3. T. *peruviana (roots and seeds, 10 g and 20 g for each)* (experiment 4).

For these set of tests, five test plots measuring approximately 1.0×0.8m were prepared in a grid of 5 blocks × 3 blocks, 20 cm apart. Test blocks of *T. scleroxylon*, measuring 10cm × 4.1cm × 2.6cm were completely buried and inspected twice at 4 week intervals over 8 weeks.

3.2.3 IDENTIFICATION OF BASIC PHYTOCHEMICALS IN PLANT SAMPLES AND EXTRACT OF T. PERUVIANA

Results obtained from the determination of antitermic properties of the pulverized materials from the various experiments indicated that *T.peruviana* root was most efficacious against termite activities. The aim at this stage was to determine the chemical composition of the pulverized materials and of extracts of the roots of *T. peruviana.* This involved the following procedures in the order indicated:

- Screening the various plant materials for the functional groups and secondary metabolites present in them
- Extraction of *Thevetia peruviana* (root)
- Further testing on the various extracts of the roots of *Thevetia peruviana*.

3.2.4 PHYTOCHEMICAL SCREENING AND FUNCTIONAL GROUP DETERMINATION

By this analysis, the presence of several phytochemicals like alkaloids, flavonoids, tannins, saponins, coumarins, carotenoids, glycosides and acids were tested (Pavia et al,1999; Carey, 2003). The functional groups like aldehydes, alcohols, esters, amides were also determined. The methods for the analysis of the various phytochemicals are described below;

SAPONINS

Powdered material (2g) was boiled in 10ml. of distilled water for 3-5 minutes. It was filtered hot and shaken vigorously. Separation or froth (foam), which persisted for some time, was indicative of saponins.

GENERAL GLYCOSIDES

About 0.5g of powdered material was put into two separate beakers and heated at 60^{o}C. 5.0ml of dilute Sulphuric acid is added to one beaker and 5.0ml of distilled water to the other. The beaker was heated on a boiling water bath for 3-5mins and the contents filtered into two separate test-tubes and allowed to cool. The cooled filtrate was made alkaline by the addition of sodium hydroxide solution and Fehlings's solution added and heated for 3 minutes. The formation of reddish-brown precipitate in the test-tube containing the filtrate from H_2SO_4 treatment and the absence of precipitate in the other test-tube indicated the presence of glycosides.

FLAVONOIDS

a) About 5ml of ethanol was poured on a small portion of the sample and filtered then a small amount of Magnesium ribbon was added, followed by concentrated hydrogen chloride drop-wise. The presence of a Brick-red colouration was an indication flavonoids.

b) A small amount of the sample was put on a filter paper and moistened with dilute ammonia solution. This was viewed under U.V light. The presence of flavonoids was indicated by a blue colouration.

TERPENOIDS AND STEROIDS

a) The powdered plant material was extracted with ethanol. About 2ml of the extract was evaporated to dryness in a crucible.

b) The dried extract was redissolved in chloroform. A few drops of acetic anhydride were added followedwithtwo drops of concentrated sulphuric acid (H_2SO_4). The presence of terpenoids was indicated if the subsequent solution turned reddish pink.

CAROTENOIDS

a) About 5g of the material was extracted with about 10ml of ethanol. To 2ml of the extract, 3ml of Antimony trichloride was added. Dark-blue colouration of the solution is indicative of carotenoids.

b) About 5g of the materials was extracted with about 10 ml of ether. Conc. H_2SO_4 (about 1ml.) was carefully added to about 2ml of the extract to form a layer under the ethereal solution. The presence or absence of an intense dark-blue or blue-violet or greenish-blue colour in the acid layer showed the presence or absence of carotenoids.

COUMARINS

About 0.2g of the powdered plant materials was taken in a test-tube and moistened with water. The test-tube was then covered with a piece of filter paper moistened with dilute NaOH solution and placed in a hot water bath of temperature about 100^{o}C. After about 15 minutes, the paper was removed and exposed to U.V light. Yellow-green fluorescence of the filter paper indicated the presence of coumarins.

ALKALOIDS

To about 5g of powdered material was added about 10ml of 1% HC1 in a test tube and left to stand in the fumed chamber for about 30 minutes with occasional stirring. It was filtered and to about 2 ml portions of the filtrate was added

a) Mayer's reagent (Potassium mercuric iodide)

b) Dragendorf's reagent (Potassium bismuth iodide)

c) Saturated aqeous solution of picric acid.

Precipitate with any of (a), (b) or (c) indicated the presence of alkaloids.

ANTHRAQUINONES

A small amount of sample was boiled with 25ml of 0.5M Potassium hydroxide and 4ml of Hydrogen peroxide The mixture was cooled, filtered and acidified with a few drops of acetic acid. The acidified mixture was extracted with a small amount of benzene (15ml). The benzene layer, which generally takes a yellow colour, was shaken with a small amount of Ammonium hydroxide. Red colouration indicates anthraquinone or colourless (alkaline) layer indicated absence of anthraquinones.

ANTHRAQUINONE GLYCOSIDES

About 0.5g powdered plant material was added to 20ml of dilute H_2SO_4 and boiled. The mixture was filtered hot allowed to cool to the feel. A portion of the cooled filtrate was shaken with an equal volume of benzene. The benzene layerwas separated and shaken with about half its volume of dilute ammonia (NH_3) solution. A colourless ammoniacal layer indicated the absence of anthraquinone glycosides.

CYANOGENETIC GLYCOSIDES

Sodium picrate paper was prepared by saturating a strip of filter paper in a solution of 5.0g Na_2CO_3 + 0.5g of picric acid dissolved in 100 ml of water. The slip was then blotted dry.

About 2.0g of finely powdered plant material was taken in a test-tube. The material was moistened with water and allowed to hydrolyze (with dil. HCl) in a stoppered test tube. A few drops of chloroform were then added and a piece of moist sodium picrate paper was inserted into the test-tube, taking care that it did not come into contact with the material or touch the inner sides of the test tube. The test tube along with its contents was kept warmed at 35^{o}C for about 3hrs. The presence of red colour of the sodium picrate paper after 3 hrs was taken as a positive test for cyanogenetic glycosides.

FUNCTIONAL GROUP DETERMINATION

Solubility tests

About 0.1g of the sample was taken into a test tube of 3 ml of distilled water and shaken vigorously. A complete dissolution of the compound indicated, it was soluble. If not it was insoluble.

The test was repeated using 3 ml of the following solvents:

a. IM NaOH
b. IM HCl
c. 85% H_3PO_4

pH test

Aquous solution of the sample was placed in a clean dry test tube and blue and red litmus papers were dipped one at a time, into the solution to wet the litmus paper. The paper was the removed and observed for any colour changes. If the solution turned blue litmus paper to red - acid was assumed to be present indicating an acidic sample. And if the solution turned red litmus paper to blue - amine was assumed to be present indicating a basic sample.

Oxidation with chromic acid

About three drops of 0.5M sodium chromate solutin ($Na_2Cr_2O_7$) was added to 1ml of 3M H_2SO_4 in a test tube. Three drops of the compound to be tested was added and shaken vigorously

For an insoluble compound, about 10 drops of acetone was added to increase the solubility, and shaken thoroughly. If the solution turned blue or green within five to ten minutes was an indication that the compound was a primary or secondary alcohol or aldehyde.

Hydrolysis of esters and amides

About 0.2g or seven drops (if liquid) of the sample was placed in a test tube and about 3 ml 6M NaOH was added and shaken vigorously.

The test tube was loosely stoppered and heated in a beaker half full of boiling water.

The stopper was removed in 10min and a moist piece of red litmus paper was carefully inserted into the vapour in the upper part of the test tube taking care not to allow the paper to touch the walls of the test tube.

The presence of an amide was indicated by the red litmus paper turning blue. In the absence of this the vapour in the test tube was cautiously smelled.

The odour of an alcohol, distinctly different from that of the original compound was an indication of the presence of an ester.

Oxidation with Benedict's solution

A solution of about 0.1g of the sample in 2 ml of water was put in a test tube and about 2 ml of Benedict's solution was added. In the absence of a colour change, the mixture was heated in boiling water bath of about 100^{o}C for 5-10 min.

Red, yellow or yellowish green precipitate of (copper (1) oxide) was an indication of the presence of an aliphatic aldehyde.

The colour of the precipitate depended on the nature and the amount of the aldehyde present.

EXTRACTION OF *T.PERUVIANA*

Pulverized roots of *Thevetia peruviana* (4kg) was filled in a thimble and extracted exhaustively and sequentially using 1L of petroleum ether (20-40), ethanol (96%) and distilled water in a soxhlet apparatus. The extractions were carried out for 2 hours, 8 hours and 24 hours, respectively. The three extracts were filtered through Whatman no.1 filter paper to remove all debris and unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent.

For the ethanol and water extract the solvents were removed with a rotary evaporator at reduced pressure to obtain the dry extractives. The temperature of the water bath for removing the ethanol was set at 40^{o}C and for removing the water the temperature was increased between 50^{o}C and 60^{o}C. The petroleum ether extract was concentrated by removing the solvent using the soxlet apparatus without the thimble at 35^{o}C. After every extract was concentrated, the total weight of every sample was taken.

The extractive content was calculated as follows:

Extractive content (%) = (w1-w2)/(w3×(1-m%)), in which

w1 is the total weight of the extractives and the flask (g)

w2 is the weight of the flask (g)

w3 is the weight of the samples weighed for extraction (g)

m% is the moisture content of the sample

All crude extractives were stored at 5^{o} C in a refrigerator. In a few cases ethanol extracts were left standing for two weeks and these yielded crystals. Recrystallization, melting point determination, and polarimetry were conducted so as to identify the crystal.

3.2.4.1 PHYTOCHEMICAL SCREENING OF EXTRACTS

Basic phytochemical screening was carried out on all the three extracts to determine the phytochemicals present in them. Procedures were same as those used above (i.e. phytochemical screening of pulverized plant samples) Stock solutions were prepared by dissolving known weight of the dried extract in 100ml each of the three solvents. Parts of these stock solutions were taken and diluted with the various solvents to prepare 2mg/ml of test solutions. For the repellency/attractancy test a concentrated ethanol was partitioned between MeOH and Cyclohexane and the MeOH-soluble fraction was subsequently partitioned with $CHCl_3$ and H_2O. The respective fractions were evaporated to dryness in vacuo to give residues as methanol, cyclohexane, chloroform and water soluble fractions. Stock solutions were prepared of known weights in 100ml of the four solvents and parts of these were diluted with the respective solvents to prepare 2mg/l of test solutions.

3.2.5 IDENTIFICATION OF MOST EFFICACIOUS EXTRACT

At this stage, bioassays were conducted in the laboratory and on the field to identify the most efficacious extract. Bioassays conducted included evaluation of extract toxicity in a force-feed environment, Repellency/attractancy test and field testing of the stakes impregnated with extracts.

Laboratory testing of extracts (evaluation of extract toxicity in a force-feed environment)

Test solution (1ml each containing 2mg of extract) was topically applied to cover the whole of 5cm filter paper. The solvent was allowed to evaporate and the filter papers were then moistened with 1ml portions of distilled water. These were placed in Petri dishes of 5cm diameter and 25 termite workers were counted onto the filter paper and covered with a mesh. Filter papers that had been treated with solvent alone served as controls. Termite mortality rates were monitored for 2 days.

Repellency Test (assayed termite attractancy or repellency to extract)

Cellulose pad halves were treated with 0.5 ml aliquots of methanol, chloroform and water solutions of *T. peruviana* extracts. These treated halves were placed beside untreated pad halves into small containers. Ten termites were added to each. The locations of the termites were noted at eight time intervals: 15, 30, 45, 60, 90, 120, 180, and 240 minutes. Based on the number of termites which chose to stay on the extract-treated pad halves, each extract was designated to be an attractant or a repellent. Extract attractancy is evinced by more than 50% of the termites remaining on extract-treated pad halves, while extract repellency is shown by less than 50% of termites staying on untreated pad halves. The test was replicated three times.

Field testing of extracts

Samples of *Triplochiton sclerixylon* (Obeche) were impregnated with extracts obtained from pet ether, ethanol and water in triplicate (45 stakes in all). Solutions were prepared by dissolving 15g of each of the extract in the corresponding 1000ml solvent that was used for the extraction i.e. pet ether, ethanol and water. Controls consisted of 45 stakes treated with only pet ether, ethanol and water. The wood samples were heated in the solution for 2 hours and left standing

for 24 hours and allowed to dry in an oven at 40^{o}C and weighed periodically till they attained constant weights. Five test plots were selected and the stakes were randomly assigned to each plot of 3 rows of 6 stakes. Dursban treated stakes (15 in all) were also assigned to 5 plots about 50 m away from the main test plots (Ocloo 1975). The graveyard test lasted for 5months and was inspected 5 times at 1-month interval. Assessment was done by visual inspection.

3.3 SEPARATION AND ISOLATION COMPONENTS OF ETHANOL EXTRACT

3.3.1 CHROMATOGRAPHY METHODS

Chromatography methods were used to study and separate the components of the ethanol extract. Methods used include Thin-Layer-Chromatography (TLC) and Column Chromatography (CC).

3.3.2 THIN-LAYER CHROMATOGRAPHY

The extractives were applied to a commercially precoated silica gel plate to perform TLC (thin layer chromatography) with a flowing solvent of different solvent systems i.e. The aims of this procedure were: to identify the number of components in the extract, distinguish the difference between extract, find out how close components of each extract are and to identify solvent/ solvent systems to be used for column chromatography.

The gel plate used was Silica Gel 60F_{254}. Visualization was done under UV light, iodine vapour and spraying with concentrated H_2SO_4 and drying in the oven at 105^{o} C.

After the spots were visualized and labelled, their retention factors (Rf value) were calculated and compared. The Rf values were calculated according to the following formula:

$$\text{Rf value} = \frac{\text{Distance from the original point to the spot}}{\text{Distance from the original point to the front line}}$$

3.3.3 COLUMN CHROMATOGRAPHY

In order to isolate the bioactive compound from the crude extracts they were further fractionated using column chromatography (silica gel) Merck 70, ASTM 70-230. A cleaned, dry column (950ml) was aligned in a vertical position. A beaker was placed under the column outlet. The column was partially filled with petroleum ether. A loose plug of cotton which had been washed with petroleum ether was tamped down in to the bottom of the column. A small layer of clean white sand was placed over the cotton wool by pouring sand in to the column. The column was tapped gently to level the surface of the sand. The column was then filled with 400 ml of petroleum ether and silica gel was added carefully from a beaker, while solvent was allowed to flow slowly from the column. The column was tapped as the silica gel was added till a desired height was attained. The solvent that drained from the column during packing was re-run through the column. The sample (5g) was dissolved in ethanol and added down the side of the column to the silica gel packing. When the sample had adsorbed to the silica gel, small amount of sand was poured in to cover sample. The mobile phase was poured continuously to the top of the column by aid of a funnel. The bottom outlet of the column was opened. As the eluent (mobile phase) passed down the column, the components of the mixture began to move down the column. The eluates (fractions) were collected in separate test tubes. The following solvents were used in the order of listing shown to elute the various fractions;

Butanol/pet ether (2:1), Butanol, Butanol/Ethanol (2:1, 1:1, 1:2), Ethanol, Ethanol/ Methanol (2:1, 1:1, 1:2), Methanol, Methanol/ Distilled water (3:1, 2:1, 1:1, 1:2, 1:3) and distilled water

The test tubes were changed as the eluate after 10 ml of each fraction were collected and analysed by thin layer chromatography technique using different solvent systems. Visualization was done under UV light, iodine vapour and spraying with concentrated H_2SO_4 and drying in the oven at 105^{o}C. Fractions were collected and pooled on the basis of similar TLC results. Four fractions were collected in all and the solvent were removed using the rotary evaporator. They were then dried on silica gel and weighed. Parts of these were later used for the brine shrimp lethality test to determine the most active fraction.

Fraction 1 (1.04g) was further partitioned between petroleum ether and methanol to obtain two fractions (fractions AM1 and AM7). These two fractions were dried under Nitrogen gas and used for High Performance Liquid Chromatography.

3.4 BIOASSAY OF FRACTIONS: BRINE SHRIMP LETHALITY TEST

The brine shrimp, *Artemia salina* toxicity test was conducted according to methods described by McLaughlin and colleagues (McLaughlin et al. 1991) and the assessment of toxicity was done by methods described elsewhere (Lieberman 1999; Milhem et al. 2008). The artificial seawater was made by adding a quarter teaspoon of sea salt, 9.5g (purchased from a pet shop in London) to 250 cm^3 of distilled water. The seawater was put in a small tank and a teaspoon of brine shrimp eggs added to one side of the tank, which was covered. The other side was not covered so as to allow light that would attract the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for 48 hours to allow for the eggs to hatch.

Test tubes used were washed and dried in an autoclave. Different concentrations of ethanol extract and the isolated compound (A) were prepared, using dimethyl sulfoxide (DMSO). Only

one concentration was used for the fractions. For each of the test extracts, 20 mg was weighed in a test tube and 2 ml of dimethyl sulfoxide was added. This served as a stock solution of concentration 10,000 ppm. For the ethanol extract and the isolated compound A, lower concentrations were prepared by using a micro pipette 0.005, 0.05 and 0.5 ml of the stock were transferred into test tubes labelled 10, 100 and 1000 ppm respectively. Artificial sea water was added to make up the test solutions to 5ml. Each test solution was replicate three times and Brine Shrimp larvae (nauplii, 10) were added to each test tube. The brine shimp tests were left for twenty-four hours, after which the number of deaths out of the 30 shrimps per dose was recorded, with the aid of a hand-lens.

3.5 SEPARATION AND ISOLATION OF ACTIVE INGREDIENTS

This involved preparation and purification of pulverized root and crude ethanol extract. The purified root, crude ethanol extract and fractions AMI and AM7 were purified using Analytical HPLC and Preparative HPLC.

3.5.1 PREPARATON OF GROUND ROOTS AND ETHANOL EXTRACT

One hundred milligrams of well pulverized *T. peruviana* roots and ethanol extract were weighed into separate 10-ml. glass-stoppered Erlenmeyer flasks. Five millilitres of 80% ethanol were added, and the samples were placed in a water bath at 70 ^{0}C for 10 minutes with constant swirling. After that time, the flask was tightly stoppered and shaken for 1 hour in an automatic shaker at room temperature. Water, 20 ml was added to the sample and the solution was transferred into a 60-ml separatory funnel and extracted five times with 4 ml aliquots of chloroform. Each extract, was filtered through a filter system made of a small funnel, 3 cm. in diameter, fitted with Whatman No.1 filter paper containing about 3g of anhydrous sodium sulphate (as a drying agent). The filtrates were collected in a 25-ml volumetric flask. The volume was made up to the mark with chloroform, which was poured through the same filter. A 10-ml. aliquot each was transferred into a 60-ml. volumetric flask and evaporated to dryness under a stream of air. These were labelled A2 and A3 corresponding to the purified root and purified ethanol extract respectively.

3.5.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography (both analytical and preparative) were conducted on the fractions (AM 1 and AM 7) the purified root (AM 2) and ethanol extract (AM3); the number of components, how close they are to each other and to separate them to their respective component.

3.5.2.1 ANALYTICAL HPLC

The Analytical HPLC setup consisted of the following;

1. PE series 200 DAD
2. Applied Biosystems 783 programmable Absorbance Detector
3. Quaternary LC Pump Model 200Q/410
4. BDS Hypersil C 18 4.6 × 25cm column

Chromatographic conditions adopted for the analytical HPLC analysis are:

1. Column: BDS Hypersil C 18 4.6 × 25cm;
2. Flow rate: 1ml/min
3. Detector / Sensitivity: UV-visible detector: 220nm
4. Diluent: 50 methanol : 50 water
5. Mobile phase: 50:50 Acetronitrile : Water
6. Injector Volume: 1µl
7. Temperature: Room temperature

About 1mg of each of the samples was dissolved in 1ml of the diluents (50ml methanol : 50 water) and 20µl of the resulting solution was injected into the analytical HPLC system. The corresponding peaks of the individual components within the sample were produced by the chromatograph.

3.5.2.2 PREPARATIVE HPLC:

The preparative HPLC system consisted of the following:

1. A Gilson HPLC chromatography system(USA)
2. Gilson pump
3. Gilson 115 UV detector
4. Column is Zorbax ODS 21.2 × 250mm column (Rockland Technologies Inc., USA)

Chromatographic conditions adopted for preparative HPLC analysis:

1. Column : Zorbax ODX 21.2× 250mm
2. Flow rate : 15ml/min
3. Detector/ Sensivity : Gilson 115 UV detector 220nm
4. Mobile phase: Acetronitrile: Water 50:50
5. Injector Volume: 50µl
6. Temperature: RT

About Img the sample was dissolved in 1ml of methanol and 50µl of the resulting solution was injected into Preparative HPLc set up. The corresponding peaks of the individual components within the sample were produced by the chromatograph and the various components collected in conical flasks for further spectroscopic analysis. Portions of the various components were evaporated to dryness using the rotary evaporator. Compound A was obtained from AM1 and Compound B was obtained from AM7.

3.6 IDENTIFICATION OF COMPONENTS

This phase involved identification of compound A and Bs using the following spectrometric methods.

3.6.1 MASS SPECTROMETRY

The mass spectrometer system (Clayton. et al. 1966) consisted of the following:

1. Waters Platform 11 Quadruple
2. Electrospray source (Manchester, England)
3. Cone Voltage: 30 V
4. Nebulising Gas: Nitrogen
5. Carrier solvent: Methanol.

Compounds A and B in solution (from the preparative HPLC) were auto sampled and ionized to cations by loss of an electron from the electrospray source. The ions were sorted and separated according to their mass to charge. The separated ions were then detected and tallied and the results were displayed on a chart.

3.6.2 INFRARED (IR) SPECTROPHOTOMETRIC ANALYSIS

About 1mg of the solid samples (compounds A, B and C) were finely ground in a small mortar with about 10 times its bulk of pure potassium bromide and the mixture pressed into a disc using a special mould and a hydraulic press. The functional group was determined using FTIR-820IA single beam laser Shimadzu Infrared Spectrophotometer.

3.6.3 ULTRA VIOLET ANALYSIS

The instrument used was UV mini-1240UV- VIS Spectrometer manufactured by Shimadzu of Kyoto, Japan.

Settings made as routine check were as follows:

a) Spectral Bandwidth 5nm
b) Wavelength accuracy ± 1.0nm.
c) Wavelength range 190- 800nm.
d) Cuvettes used Precision cells made of quartz suprash.10mm Path length, Ω 3.5ml volume
e) Absorbance Range 0.00-0.80A
0.00-1.00A

The samples were prepared as a 0.1% solution in Chloroform. Chloroform blank was scanned at the set parameters to correct the baseline after which the Chloroform solution of the sample was scanned at the stated measurement parameters. The spectrum generated appeared on the LCD screen of the instrument and a hard copy was obtained by printing on an EPSON FX- 870 printer.

3.6.4 NUCLEAR MAGNETIC RESONANCE

About 5mg- (0.005g of each product (Compound A, B and D)) was weighed into a small vial. This was dissolved in 0.75ml of deuteriochloroform ($CDCl_3$).This solution which was free from any undissolved solid was transferred into a clean dry NMR tube and capped. To achieve this, a glass Pasteur pipette was prepared for use as a filter. This was done by pushing a small plug of cotton into the constriction of the Pasteur pipette and placing a clean dry NMR tube under it. The solution to be filtered was then added to the Pasteur pipette and the filtered through the cotton wool.

^{1}H NMR, ^{13}C NMR and HMQC were recorded on a Bruker 400MHz Spectrophotometer (^{1}H 400 MHz and ^{13}C 100 MHz) using TMS as internal standard for both nuclei. Chemical shifts (d) were given in ppm and *J* couplings in Hertz (Hz).

3.7 PREPARATION OF DERIVATIVE

Compound D was obtained by the acidic hydrolysis of a portion of Compound A (375 mg) was dissolved in methanol (MeOH) (125 ml) under sonication, followed by addition of aqueous 1 mol L^{-1}hyrogen chloride solution (125ml). The solution was heated at 55ºC, for 35 min, followed by extraction with chloroform (3 × 250 ml). The organic layer was neutralized with 3% sodium hydrogen carbonate ($NaHCO_3$) aqueous solution and concentrated until residue (147.85 mg). Portions of the residue (20 mg) were dissolved in MeOH (1 ml) for injection into GC-MS equipment. Four strong peaks were obtained, analysed and had good matches. 5mg of compound D was also used for infra red analysis as indicated in Section 3.8.2 dimethyl sulfoxide above.

SAMPLE PREPARATION FOR GC-MS ANALYSIS

About 5mg of compound D was dissolved in 1ml of methanol giving a final concentration was 0.5mg/ml.

INSTRUMENT CONTROL PARAMETERS: Agilent 5975C

Control Information

Oven	
Equilibration Time	2 min
Oven Program	On 150 °C for 2.5 min then 20 °C/min to 320 °C for 15 min
Run Time	26 min
Injection Volume	1 µL
Front SS Inlet He	
Mode	Splitless
Heater	On 250 °C
Pressure	On 7.0699 psi
Total Flow	On 52 ml/min
Septum Purge Flow	On 1 ml/min
Gas Saver	On 15 ml/min After 2 min
Purge Flow to Split Vent	50 ml/min at 0.5 min
Thermal Aux 2 [MSD Transfer Line]that Heater	On
Temperature Program	On 1 ml/min
320 °C for 0 min	
Run Time	26 min
Column #1	
HP-5MS 5% Phenyl Methyl SiloxHP-5MS 5%	
Phenyl Methyl Silox	325 °C: 30 m x 250 µm x 0.25 µm
(Initial)	150 °C
Pressure	13.332 psi
Flow	1 ml/min
Average Velocity	38.051 cm/sec
Holdup Time	1.314 min
Flow Program	On 1 ml/min for 0 min
Run Time	26 min
MS ACQUISITION PARAMETERS	
Solvent Delay	4 min
EMV Mode	Relative
Relative Voltage	153
Resulting EM Voltage	1376
Scan range	40.0-450.0
Threshold	49:59:00
MS Source	230 C
MS Quad	150 C

3.8 OTHER ANALYSES

In a few cases ethanol extracts were not refrigerated but were left standing for two weeks and crystals were formed. Recrystallization, melting point determination, and polarimetry were conducted so as to identify the crystal. This was labelled compound C.

3.8.1 POLARIMETRY

About 2g of the crystal (Compound C) was and dissolved in about 30 ml of distilled water in a beaker. The solution was carefully transferred into a 50 ml volumetric flask and the beaker washed several times with small amounts of distilled water and all the washings added to the solution in the volumetric flask. The solution was diluted to 50 ml with distilled water and thoroughly mixed.

The polarimeter tube was filled with the blank (distilled water), taking care that no air bubbles remain in the tube. The zero reading for the polarimeter was found. The solution was transfered to the polarimeter tube, making sure that no air bubbles remain in the tube. The rotation, α of the solution is the difference between the polarimeter reading of the sample and the zero reading. The solution was transferred from the polarimeter tube back into the volumetric flask and two drops of 0.01 M HCl added and mixed thoroughly. The rotation of the acidified solution was determined at 5 minutes intervals until an equilibrium value was obtained.

3.9 DATA ANALYSIS

The principal analyses of data generated involved first the descriptive analysis of the antitermitic properties of study plant products and statistical analysis of the efficacy of the study plant products.

The antitermitic properties of test plant samples assessed included toxicity to termites, repellency and attractancy and resistance to termite destruction. To determine repellency, the number of termites on each extract-treated filter paper and on each solvent-treated filter paper (control) after 240 minutes of observation was counted and the mean of three replicates determined and compared. The average counts were then converted to percentage repellency (PR) using the formula: ***[PR = 2(C – 50)]*** (Talukder and Howse 1993; Talukder and Howse 1995) where C is the percentage of termite on the untreated filter paper. A positive PR indicated repellency while a negative PR indicated attractancy. The percentage repellency caused by various samples was plotted against time for comparison (Fig 4-11).

Resistance to termite destruction was assessed in two ways: (1) weight loss following exposure and (2) visual assessment of termite attack and damage.

The average weight loss of test blocks following exposure to plant materials was determined by subtracting average final weights from average initial weights. These data were used to compute the percentage weight loss using the equation:

%WL = (IW-FW) X100/IW; where IW is the average initial weight and FW is the average final weight. The percentage weight loss was then compared among the plant materials using analysis of variance (ANOVA) and multiple regression analyses.

A visual assessment of termite attack and damage was done by adapting a damage rating system recommended by Pearce 1997 (Table 3-3) as a measure of resistance to termite destruction (Pearce 1997). The degree of attack and destruction to the test blocks were scored 0 through 5 and each score was assigned percentages from 0 to 100%. Each

assessment was replicated three times on each plot. The scores were entered in MS Excel spread sheet programmed to calculate the mean score from the replicates per plot and the appropriate percentage was assigned to each mean score. The Percentage mean scores were transformed using log (x + 1) to correct for normality. The data was then analysed using one-way ANOVA.

Table 3-3: Termite damage ratings and their corresponding descriptive indicators for on-farm testing of plant materials adapted from Peace, 1997.

Damage rating	Descriptive indicator (s)	Scoring	Damage rating (%)
1	No attack	0	0
2	Attempted attack; Superficial gnawing or nibbling with insufficient depth to be measured	1	20
3	Slight attack; Some definite surface attack or small holes less than 3 mm	2	40
4	Average attack; Surface attack (< 1 mm) deep in places or spread out over most of the sample, holes greater than 3 mm but no cavities	3	60
5	Strong attack; more than a quarter of the surface eaten or cavities in specimen.	4	80
6	Total destruction	5	100

Finally a description of the chemistry as obtained from various experiments i.e. phytochemical screening, chromatography and spectrometry to aid the chemical characterization of the active component of the most efficacious plant product.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

This chapter presents the results and discussion of the analyses of data generated during experiments carried out in this study relating to the antitermitic efficacy of the experimental plants and the chemical characterization of the active component of the most efficacious plant.

4.1 ANTITERMITIC EFFICACY

This section provides the analyses of the assessment of the antitermitic efficacy of the experimental plants based on their resistance to damage by termites, repellency and toxicity and discusses the outcomes in relation to the basic phytochemicals found.

4.1.1 TERMITE SPECIES ON TESTING SITE

The wood feeding termite species found at the Fumesua termite testing included; *Ancistrotermes cavithorax* (Sjostedt), *Ancistrotermes crucifier* (Sjostedt), *Ancistrotermes guineeensis* (Silvestri), *Odontotermes pauperans* (Syvestri), *Coptotermes intermedius* (Sylvestri), *Macrotermes bellicosus* (Smeathman), *Pseudocanthotermes militaris* (Hagen) and *Macrotermes subhyalinus* (Rambur). This finding agreed with that reported earlier by Usher that these species are abundant at this site (Usher 1978).

4.1.2 RESISTANCE TO TERMITE DAMAGE

Resistance to termite destruction was assessed in two ways: (1) weight loss following exposure and (2) visual assessment of termite attack and damage.

4.1.2.1 MEASURING AND COMPARISON OF WEIGHT LOSS

An initial exploration of the data showed that change in weight was normally distributed. Analysis of variance (ANOVA) and multiple regression analyses of the absolute loss in weight from baseline weights over time during which wood stakes were buried in soil mixed with plant materials and those impregnated with plant extracts were carried out. The pattern of differences in weight loss among medians and means are similar across samples rejecting the null hypothesis of equal means and variance (Figure 4-1) and also indicated by a low Bartlett's probability (p=0.001).

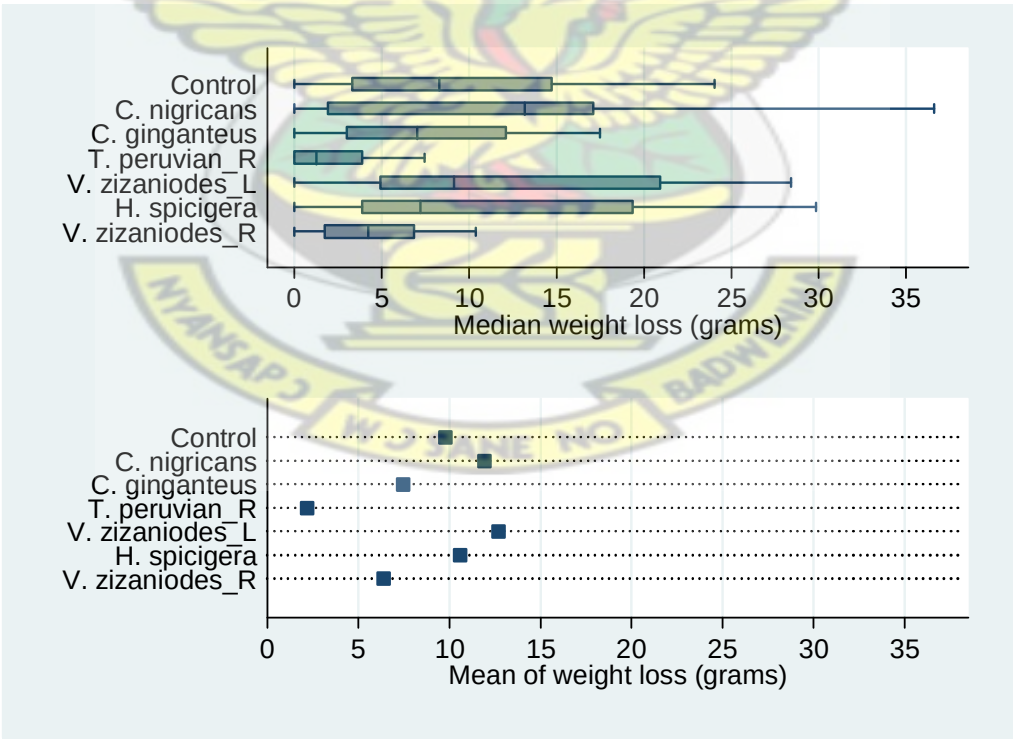


Figure 4-1: Patterns of differences in weight loss among medians and among means

A pair wise correlation showed significant correlation between initial and final weights of wood samples and change in weight ($p<0.001$). This is also confirmed by linearity between the change in weight and type of sample (Figure 4-2). The effect of type of test plant on the mean changes in weight after exposure to termites was determined in a multivariate regression analysis.

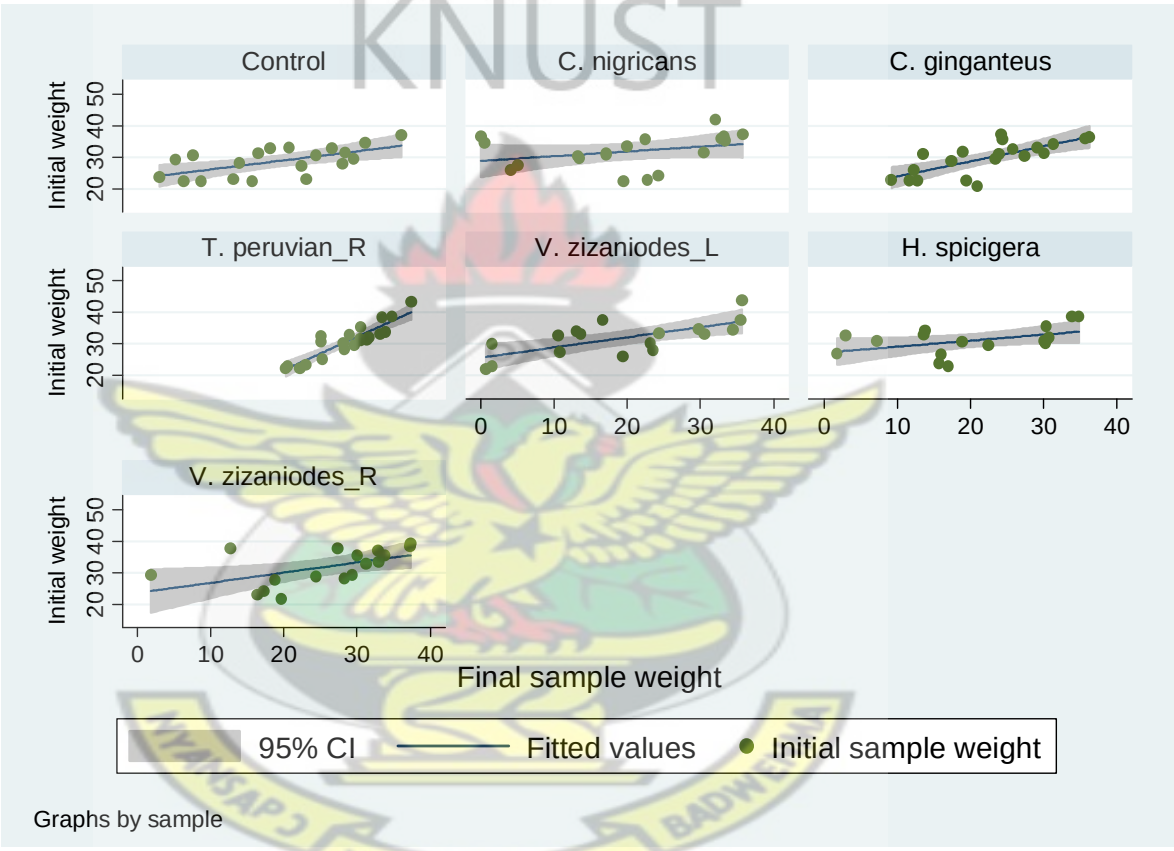


Figure 4-2: Linearity between change in weight and type of plant material

The effect of soil treatment was independent of the effect of plot and the initial weight of stake. (Table 4-1 and Figure 4-3). As shown in Figures 4-3 and 4-4, there was a general decrease in weight of wood samples exposed to study plants over time. However, the

pattern of decrease was significantly less in soils mixed with materials from *T. peruviana* compared to the other treatments on every plot.

There was an overall mean loss in weight of 8.6gm (95% CI; 7.1 to 10.0 p<0.001) in all wood samples exposed to termites regardless of soil treatment. As shown in Table 4-1, the decrease in weight was associated with the type of plant material applied (F-statistic = 4.13; p=0.001). A comparison of the test plant materials with the control sample showed that weight loss was less in *T. peruviana, C. giganteus* and root of *V. zizaniodes* respectively compared to the control sample but only *T. peruviana* significantly (p=0.003) resisted termite damage and loss in weight. The level of loss was not significantly affected when the effects of the different plots on which the samples were buried were adjusted for in the regression model.

Table 4-1: Comparison of change in weight of buried stakes by type of soil treatment

Sample	Mean percentage weight loss (%)	SD[1]	Unadjusted LSD[2] (95% Conf. Interval)		P-value	Adjusted[3] LSD (95% Conf. Interval)		P-value
Control	35.5	28.9						
C. nigricans	37.3	34.2	1.8	(-15.2 - 18.7)	0.84	1.1	(-15.1 - 17.2)	0.90
C. giganteus	26.1	20.0	-9.4	(-26.1 - 7.3)	0.27	-9.6	(-25.5 - 6.4)	0.24
T. peruviana	6.6	7.0	-28.9	(-45.4 - -12.3)	0.001	-28.8	(-44.6 - -13.0)	<0.0001
V. zizanioides	42.7	32.9	7.2	(-10.3 - 24.6)	0.42	8.4	(-8.2 - 25.1)	0.32
H. spicigera	34.7	31.1	-0.8	(-18.3 - 16.6)	0.93	-0.9	(-17.6 - 15.7)	0.91
V. zizanioides	20.4	25.3	-15.1	(-32.5 - 2.4)	0.09	-13.8	(-30.5 - 2.8)	0.10

[1] Standard deviation
[2] Least square difference

[3] LSDs adjusted for the effect of plots

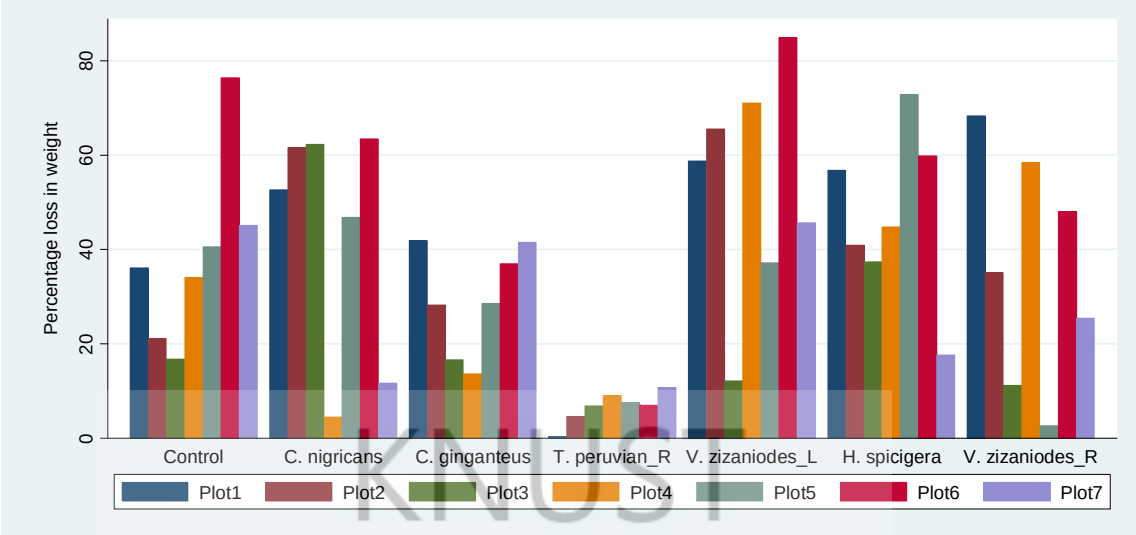


Figure 4-3: Susceptibility to termite damage measured by change in weight of buried stakes.

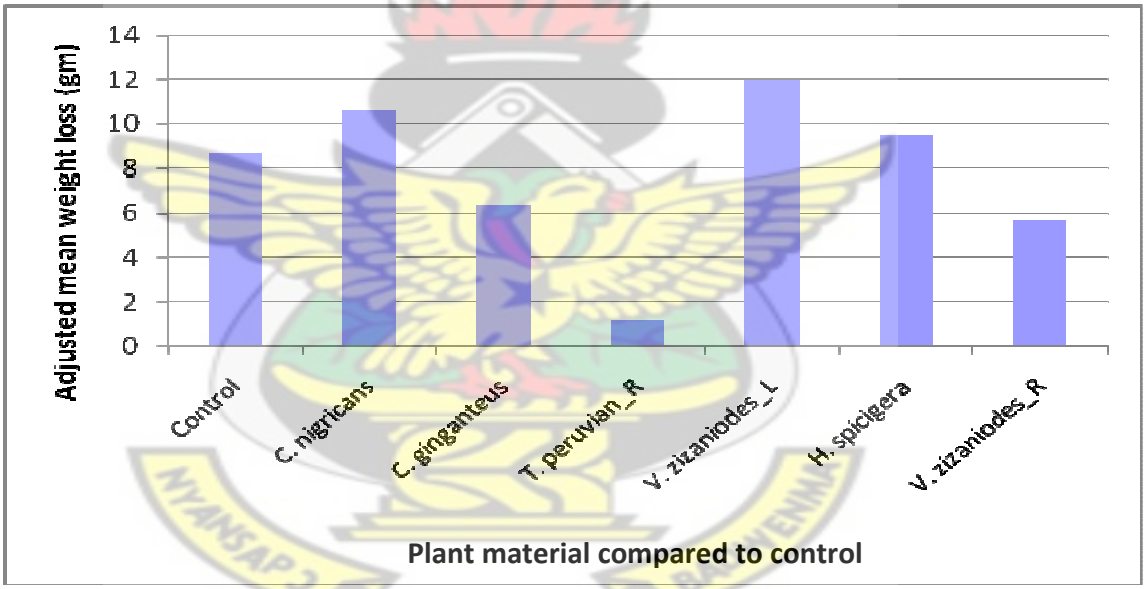


Figure 4-4: The mean loss in weight in grammes adjusting for the effect of initial weight of stakes and the plots on which they were burried.

4.1.2.2 VISUAL ASSESSMENT OF TERMITE ATTACK AND DAMAGE

During the field assessment of resistance to termite, varied degrees of termite and plant material interactions were observed and rated using a damage rating system adapted from the one originally recommended by Pearce, 1997. In all cases *T. peruviana* was observed to be the least susceptible to termite destruction and suffered the least termite attack (Table 4-2, 4-3, 4-4, 4-5). Stakes buried in soils mixed with *C. nigricans, C. ginganteus, V. zizaniodes and H. spicigera* and those buried in the untrated soil (control) soil were found to be severely attacked and damaged by termites on the various plots. Those stakes were completely covered by termite feeding tunnels.

Table 4-2 shows the comparison of the susceptibility of *Thevetia peruviana* to termite destruction with those of *C. nigricans, C. giganteus, V. zizanioides, H. spicigera, J. curcas, C. odorata* and *C. procera* (F =15.639, df = 6, 140, p = 0.0001; appendix 18).

Table 4-3 compares the susceptibility of *Thevetia peruviana* to termite destruction with those of *C. odorata* and *C. procera* (F =3.165, df = 4, 70, p = 0.019; appendix 19).

Table 4-4 compares the susceptibility of *Thevetia peruviana* to termite destruction with that of *J. curcas* (F = 2.903, df = 3, 56, p = 0.043; appendix 20).

In soils mixed with the seed and roots of *T. peruviana* of different weights (10g and 20g) stakes buried in soils mixed with 10 gm of pulverised *T. peruviana* root also resisted termite attack completely (F = 2.903, df = 3, 56 p = 0.0427; Table 4-5; appendix 21).

The termite resistance property of *T. peruviana* seemed to be enhanced by ethanol as shown in Table 4-6. *T.peruviana* compares very well with Dursban after 5 months of field exposure. (F = 2855.9 df = 6, 98, p = 0.0001; Table 4-6; appendix 22).

Table 4-2:Expt 1- Termite damage on *T.scleroxylon* stakes buried in soil mixed with plant material after 2 months

Sample	Mean Percentage Damage / Sig
H. spicigera	57.1429 / a
V. zizanioides (leaf)	56.1905 / a
C. nigricans	54.2857 / a
C. giganteus	53.2381 / a
V. zizanioides (root)	44.7619 / a
Control	44.7619 / a
T.peruviana (root)	12.381 / b

Table 4-3: Expt 2- Termite damage on *T.scleroxylon* stakes buried in soil mixed with plant material after 2 months

Sample	Mean Percentage Damage / Sig
C. Odorota (stem)	42.67 / a
C. procera	42.67 / a
C.. Odorota (leaf)	36.00 / ab
Control	24.00 / b
T.peruviana (root)	17.33 / b

Table 4-4: : Expt 3- Termite damage on *T.scleroxylon* stakes buried in soil mixed with plant material after 2 months

Sample	Mean Percentage Damage / Sig
Jatropha (root)	17.3333 / a
Jatropha (seed)	9.3333 / a
Control	6.6667 / a
T.peruviana (root)	2.6667 / b

All Means followed by same letter are not significantly different

Table 4-5: Expt 4- Termite damage on *T.scleroxylon* stakes buried in soil mixed with *T. Peruviana* after 2 months

Sample	Mean Percentage Damage / Sig
T.peruviana (20g root)	8 / a
T.peruviana (10g root)	0 / a
T.peruviana (20g seed)	8 / a
T.peruviana (10g seed)	29.3333 / b
control	33.3333 / b

Table 4-6: Termite damage on *T.scleroxylon* stakes impregnated with extracts of *T. Peruviana* after 5 months

Sample	Mean Percentage Damageq / Sig
Water only	74.67 / a
Pet ether extract	60.00 / a
Pet ether only	58.67 / a
Ethanol only	54.67 / a
Water extract	53.33 / a
Ethanol extract	0.00 / b
Dursban	0.00 / b

All Means followed by same letter are not significantly different

4.1.3 TOXICITY TO TERMITES

This was measured in terms of number of termites dying following exposure to study plant products in the laboratory. There were varying degrees of termite mortality when termites were exposed to different parts of various test plants in the laboratory over time (Figures 4-5, 4-6 and 4-7). Apart from the control material, all plant materials caused increased termite mortality with time.

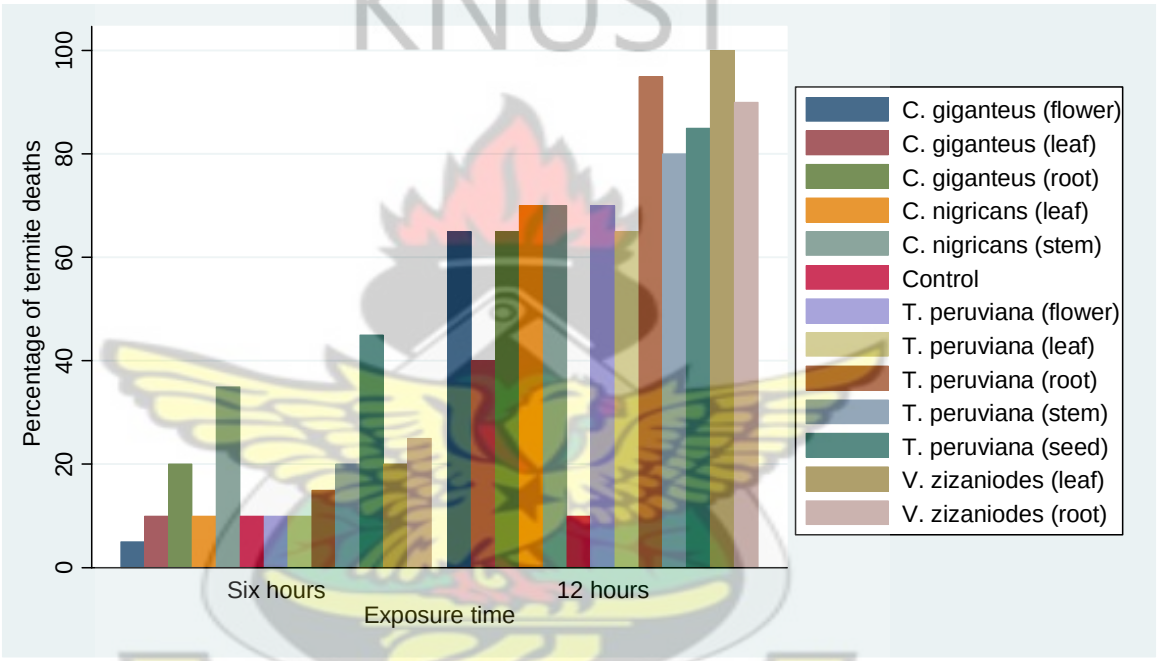


Figure 4-5: Termite mortality after 6hrs and 12hrs exposure to test plant products

However, contact with ethanol extract of *T. peruviana* caused the highest (i.e. 97%) mortality followed by the petroleum ether and water extracts in that order. Termite mortality in the control was under 10% (Figure 4-6). A total of 175 termite mortality occurred when they were exposed to extracts of *T.peruviana*; 15, 51, 81 and 28 in the control, petroleum extract, ethanol extract and water extract respectively (figure 4-7). The differences in mortality regardless of exposure duration as compared by symmetry and

marginal homogeneity tests (appendix 23) showed statistically significant differences ($P < 0.0001$) between the extracts.

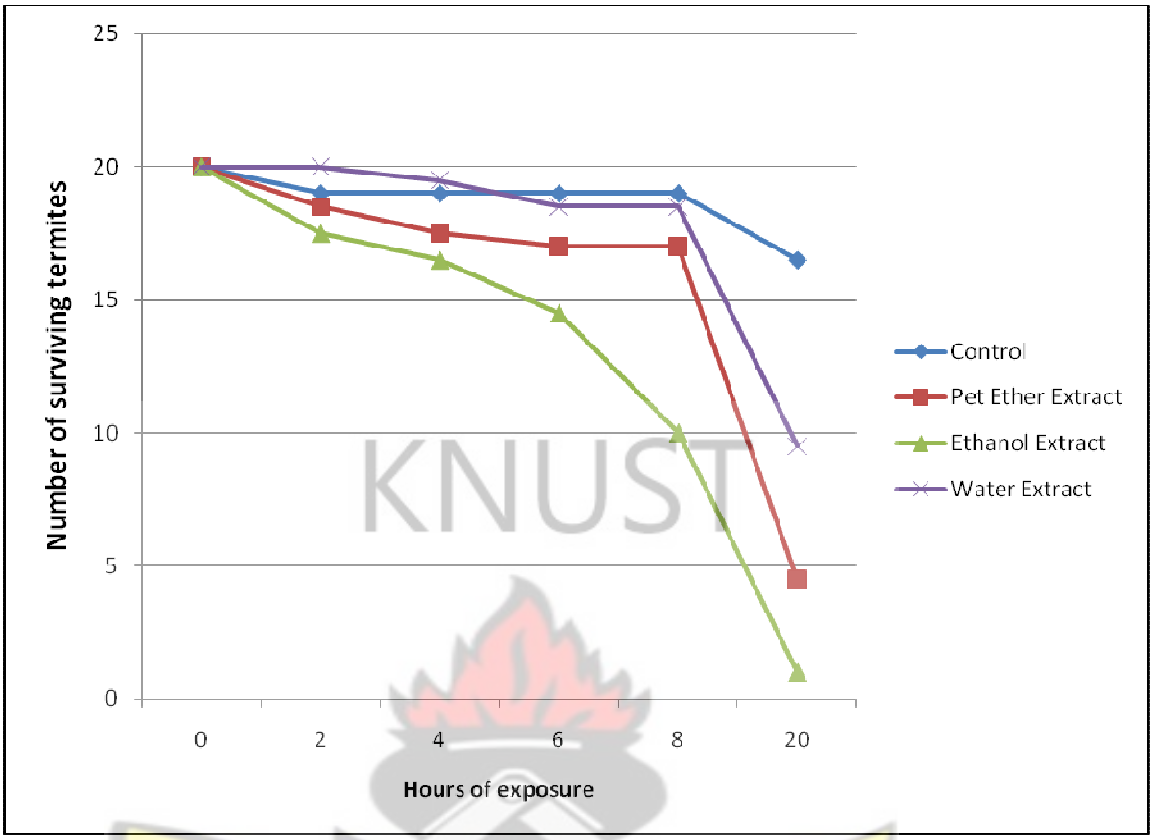


Figure 4-6: Survival of termites over 20 hr period of exposure to T. peruviana extracts

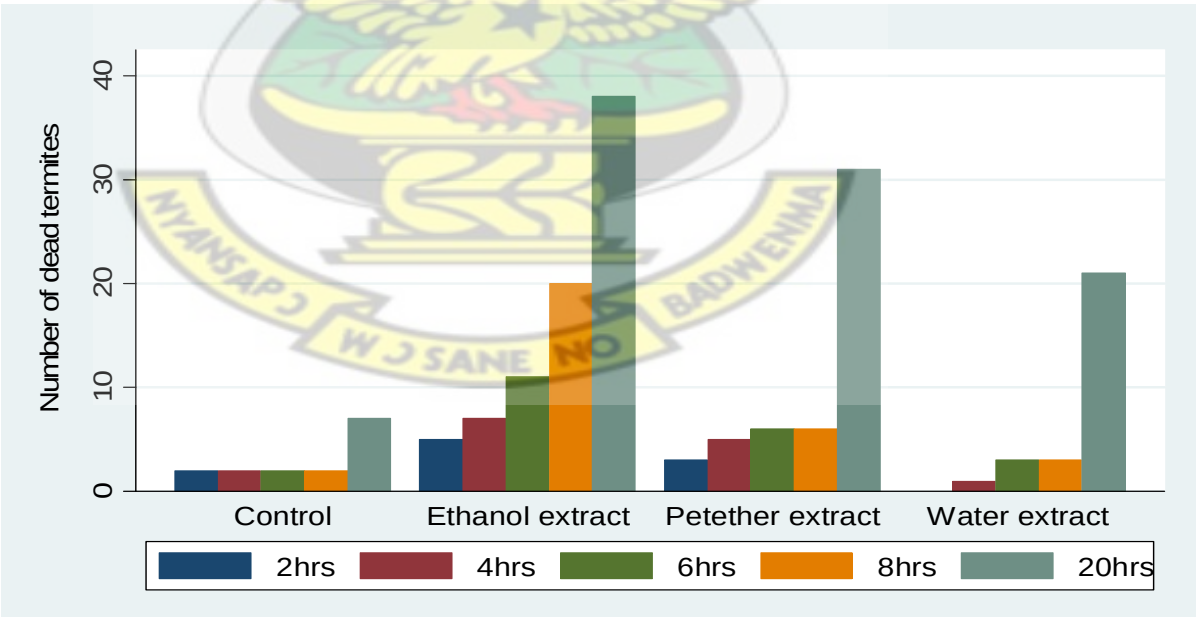


Figure 4-7: Cumulative deaths of termites over 20 hr period of exposure to *T. peruviana* extracts

4.1.3.1 BRINE SHRIMP TOXICITY ASSESSMENT

The toxic effect of the ethanolic extracts; column chromatography fractions and purified fraction of ethanolic extracts of *T. peruviana* were assessed using the brine shrimp toxicity test (McLaughlin et al. 1991; Lieberman 1999). The results are shown in Figures 4-8, 4-9 and 4-10. Figure 4-8 shows the percentage of viable brine shrimp larvae left after exposure to crude ethanolic extracts of *T. peruviana* at varying concentrations. At both 12 and 24 hours of observation, the extract applied at lower concentrations was the least toxic and so toxicity increased with increased concentration of the extract. Figure 4-9 shows the percentage of viable brine shrimp larvae exposed to column chromatography fractions of the ethanolic extracts of *T. peruviana* surviving over time. Fraction 1 was most lethal at 12 and 24 hours respectively. Figure 4-10 shows that the isolated active component in the ethanol extract of *T. peruviana* is highly toxic to brine shrimp larvae as none survived even at the lowest concentration.

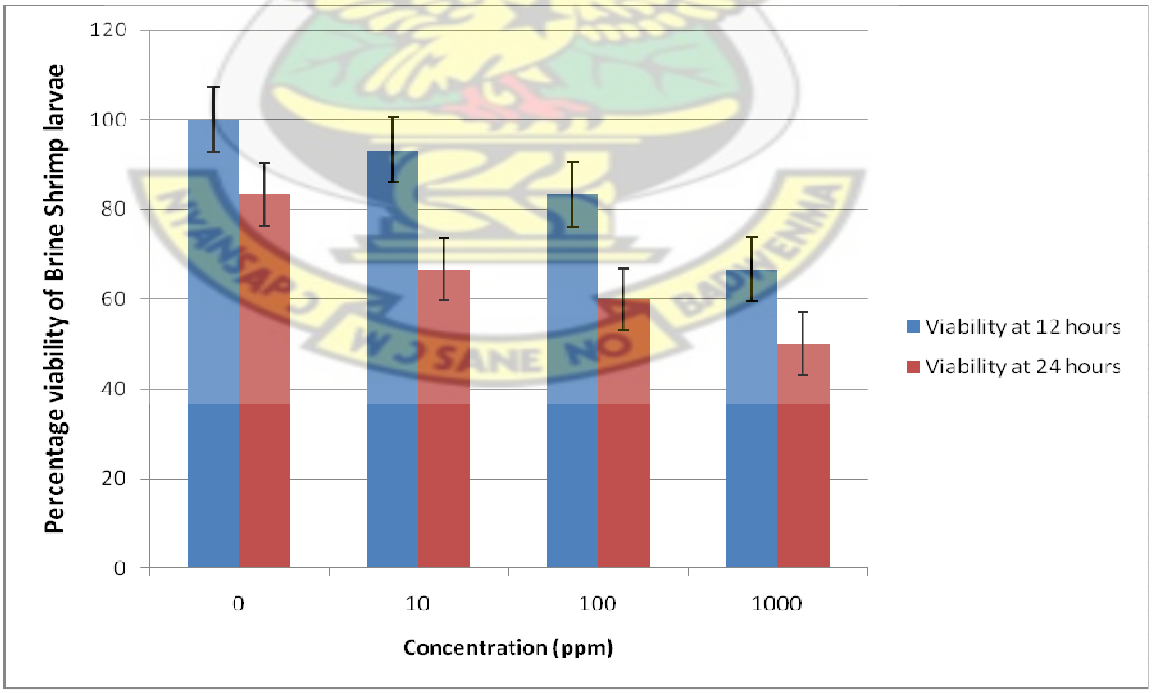


Figure 4-8: Percentage of viable brine shrimp larvae (Mean ± SD) exposed to the ethanolic extracts of *T. peruviana*

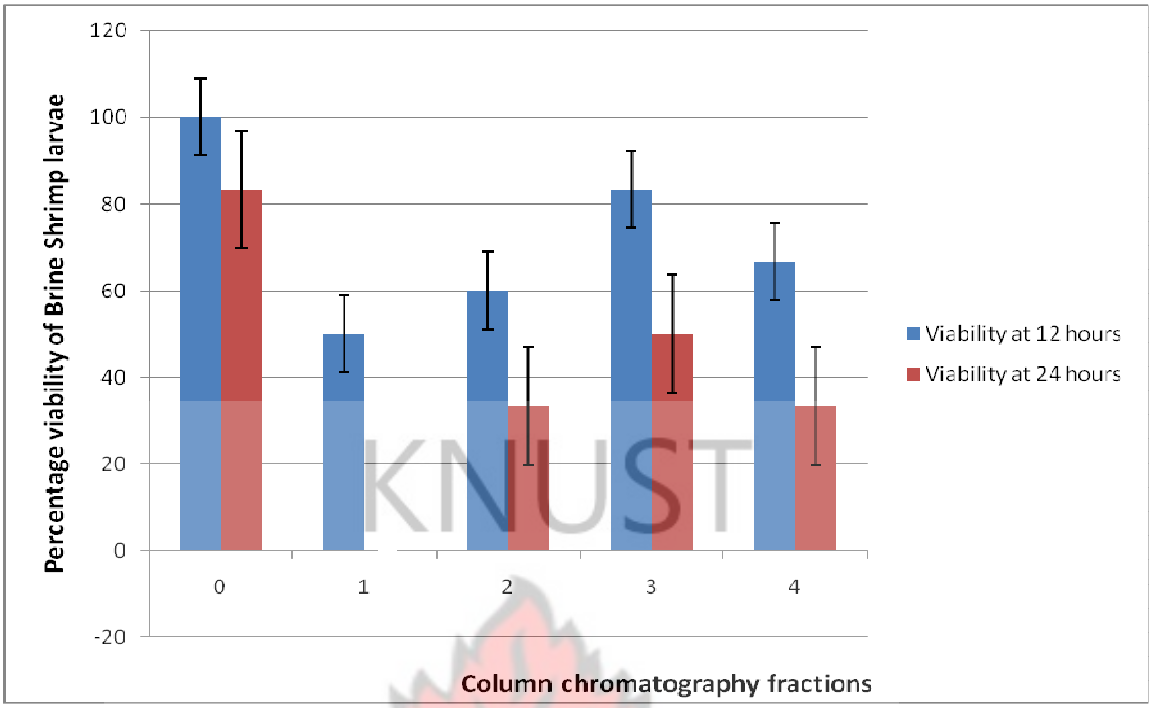


Figure 4-9: Percentage of viable brine shrimp larvae (Mean ± SD) after exposure to fractions obtained from column chromatography of ethanolic extracts of *T. peruviana*

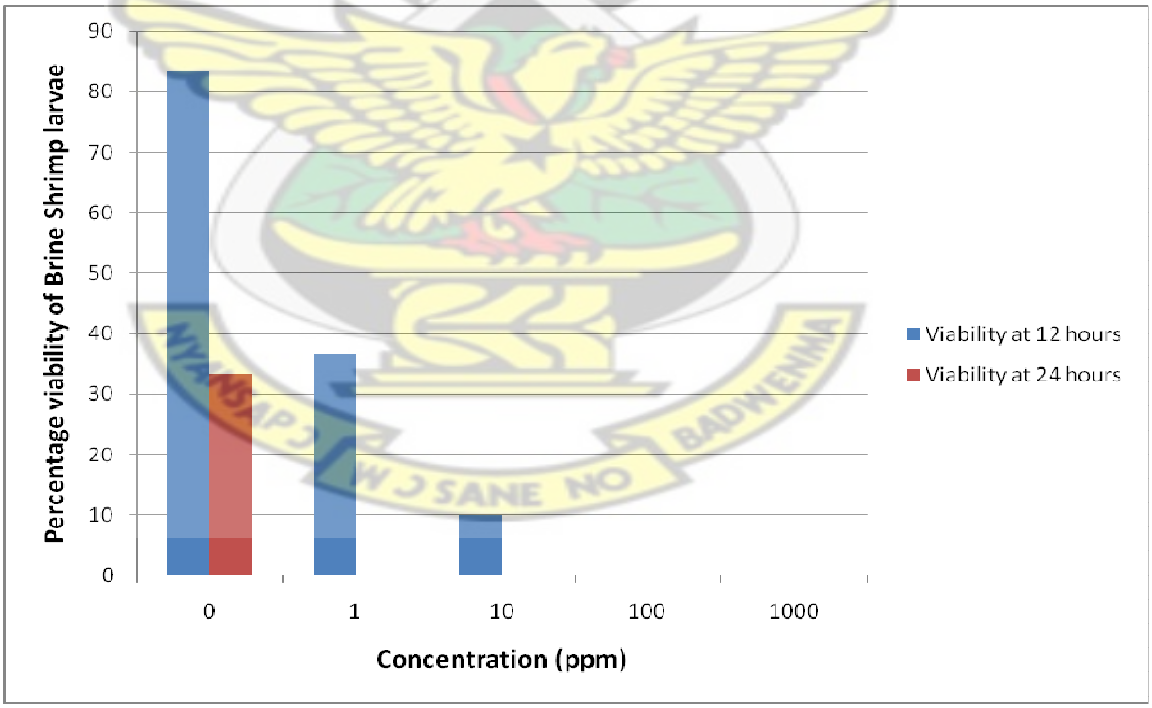


Figure 4-10: Percentage of viable brine shrimp larvae (Mean ± SD) after exposure to isolated active component of the ethanolic extracts of *T.peruviana*

4.1.4 REPELLENCY/ATTRACTANCY

During a 4 hour test period, attractancy and/or repellency of *T. peruviana* extract in chloroform, water, ethanol and methanol against subterranean termites were assessed. To determine repellency, the average number of termites on the untreated half of disc was converted to percentage repellency (PR) using the formula: [PR = 2(C – 50)] (Talukder and Howse 1993; Talukder and Howse 1995) where C is the percentage of termite on the untreated half of the disc. There was significant increase in the numbers of termites in contact with the ethanol extract, methanol fraction and methanol control discs in comparison to the numbers of termites on the corresponding untreated pads. In contrast, there was significantly less numbers of termites in contact with the chloroform fraction and water fraction discs in comparison to the numbers of termites on the corresponding untreated pads (Figure 4-11). There was no significant difference in the average number of termites present on all untreated pads (p= 0.858, 0.993, 0.834, 0.244) (Table 4-7). But there were differences in the average number of termites present on the treated pads (p=0.007, 0.003, 0.001, 0.001) (Table 4-8). The current study shows that methanol and ethanol enhanced attractancy of *T. peruviana* extract to subterranean termites.

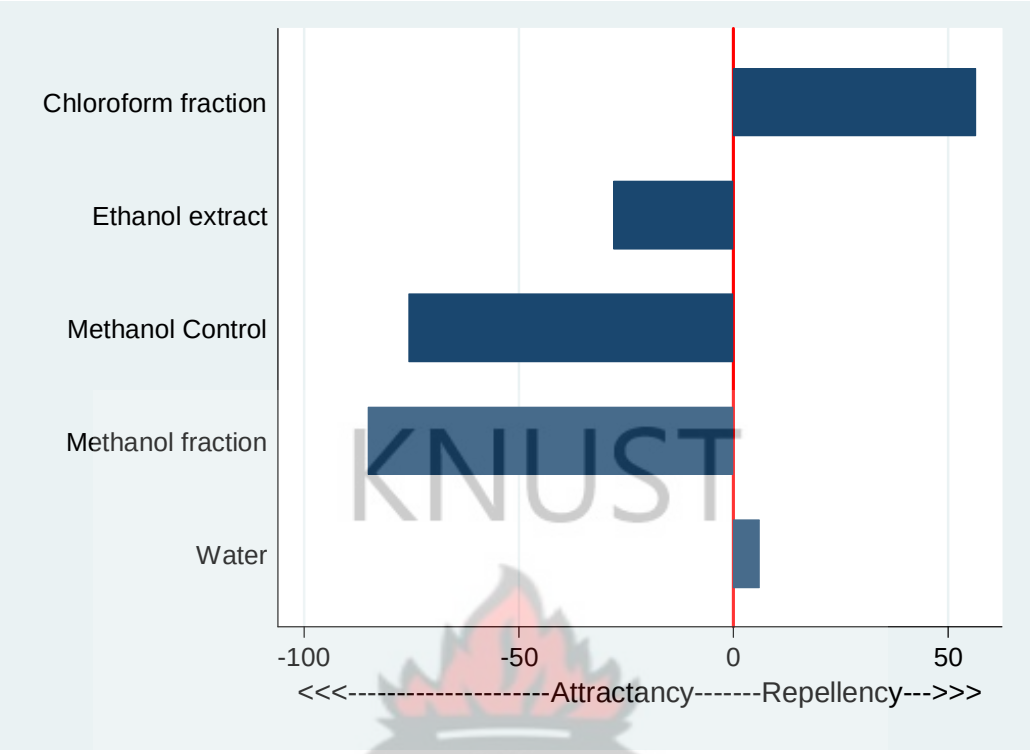


Figure 4-11: Attractancy or repellency of termites to extracts of *Thevetia peruviana* in different solvent media.

Table 4-7: Mean number of termites on untreated pads after adjusting for those on treated pads.

Sample	Mean (%) contact	SD	Coefficient of regression	[95% Conf. Interval]	p-value
Chloroform fraction	78.2	11.3	1.0		
Ethanol extract	36.1	8.8	1.3	(-13.3 - 15.5)	0.858
Methanol control	12.2	8.1	0.1	(-19.1 - 19.3)	0.993
Methanol fraction	7.5	6.1	2.2	(-18.7 - 23.0)	0.834
Water	53.1	27.8	7.2	(-5.2 - 19.6)	0.244

Table 4-8: Mean number of termite on treated pads after adjusting for those on untreated pads.

Sample	Mean (%) contact	SD	Coefficient of regression	[95% Conf. Interval]	p-value
Chloroform fraction	21.8	11.3	1.0		
Ethanol extract	63.9	8.8	14.5	(4.2 - 24.9)	0.007
Methanol control	85.9	6.4	20.9	(7.7 - 34.1)	0.003
Methanol fraction	92.5	6.1	24.4	(10.4 - 38.4)	0.001
Water	46.9	20.4	14.9	(6.4 - 23.4)	0.001

The plants and extracted materials in this study exhibited varying degrees of toxicity, attractancy, repellency and feeding deterrence against subterranean termites. *T. peruviana* had significantly higher antitermitic activity compared to the control and other test plants. It was the least susceptible to termite destruction of wawa stakes compared to the other test plants. The reason for the difference in the bioactivity of the experimental plants may be due, at least partially, to chemistry of the plants, the method of extraction, formulation and time of application, as well as termite behaviour. Since the series of field and laboratory experiments in this study were done according to predefined standard operating procedures, the method of extraction, formulation and time of application and termite behaviour are not likely to significantly influence the differences observed.

However, the differences in antitermitic efficacy of the various experimental plants as observed in this study are consistent with the view that plant chemistry strongly influences plant-termite interactions. There are two fundamental components to the role that plant chemicals may play in the mediation of these interactions; (1) definition of the plant's nutritional value to the termite and (2) formation of, or at least contribution to, the cues upon which the termite's sensory perception of the plant is based. A given plant metabolite could contribute either positively or negatively to the plant's nutritional value to the termite or it could function as a cue, or signal, on which the termite would rely in making its dietary choices (attractant or repellent).

The phytochemical screening in this study showed that all the experimental plant parts have terpenoids and steroids, and one or more of other phytoconstituents such as general glycosides, tannins, polyphenols, carotenoids, saponins and flavonoids. All these

phytochemicals have been reported in earlier works to have antitermitic activities (Trikojus 1935; Nakashima and Shimizu 1972; Chang et al. 2001; Cheng et al. 2004; Ganapaty et al. 2004; Kusumoto et al. 2009).

However, in relating the phytochemicals to the antitermitic activity of the plants one needs to interpret the role of plant chemistry in plant-termite interactions within the framework of three principles. These are (1) the molecular basis for chemical cues; (2) the molecular diversity of chemical signals which implies specific mechanisms for plant-initiated attraction or repellence of termites; and (3) there are dynamic elements to many plant chemical defences (Reichardt 1995). This might explain why the presence of these phytochemicals notwithstanding, the experimental plants in this study did not show equal antitermitic activity.

Results of phytochemical screening of the extract showed that ethanol extract of *T. peruviana* which was most efficacious in controlling termite infestation, contains glycosides. In the attractancy/repellency tests, the ethanol extract was partitioned in methanol, chloroform and water. The relatively non-polar fraction, chloroform fraction showed repellency whilst the polar fractions ethanol and methanol fractions showed attrantancy with water fraction showing just borderline repellency (Fig 4-11). Thus the observed attractancy and toxicity of the ethanol extract of *T.peruviana* may be due to the sugar moiety attached to the poisonous genin and the fact that the genin part may be soluble in non-polar solvents whilst the sugar moiety may be soluble in the polar solvents. The case of water which is expected to dissolve more sugar moiety may be due to the fact that activity of naturally occurring isolated compounds decreases with increasing amounts

of sugar residues (Ohmura et al, 1977). The case of soils mixed with *T. peruviana* of different parts and weight stakes buried in soils mixed with 10 gm and 20g of pulverised *T. peruviana* root and seed may also be due to the increasing amount of sugar residues in the soil hence a reduction in the anti-termite activity with the greater quantity of pulverised plant samples. However both attractancy and repellency properties may be employed in the formulation of antitermitic agents.

4.2 CHEMICAL CHARACTERISTICS OF *THEVETIA PERUVIANA*

This section presents the results and analyses of other processes including bioassays and phytochemical analysis of samples that led to the chemical characterisation of the active component of the most efficacious plant. The final part of this chapter presents the outcomes of the various methods including chromatography, ultra violet spectrometry, Mass spectrometry and Nuclear Magnetic Resonance spectrometry used in characterising the chemical constituents of most efficacious study plant.

Table 4-9: Phytoconstituents of parts of *T.peruviana* and other tested plants

PLANT MATERIAL	CLASSES OF PHYTOCONSTITUENT IDENTIFIED
T. peruviana (leaf)	Terpenoids, steroids, general glycosides and carotenoids
T. peruviana (stem)	Terpenoids, steroids, general glycosides, tannins and polyphenols.
T. peruviana (seed)	Terpenoids, steroids, general glycosides, tannins and polyphenols.
T. peruviana (root)	Terpenoids, steroids, general glycosides, tannins and polyphenols.
T. peruviana (flower)	Terpenoids, steroids, carotenoids, tannings and polyphenols.
C. nigricans (leaf)	Terpenoids, steroids
C. nigricans (stem)	Terpenoids, steroids, general glycosides
C. giganteus (leaf)	Terpenoids, steroids, general glycosides and carotenoids, saponins and flavonoids
C. giganteus (root)	Terpenoids, steroids, saponins alkaloids tannins and polyphenols.
C. giganteus (flower)	Terpenoids, steroids, saponins and general glycosides.
V. zizanioides (leaf)	Terpenoids, steroids, tannins and polyphenols
V. zizanioides (root)	Terpenoids and steroids
Carapa procera (bark)	Tannins , glycosides, terpenoids
Jatropha curcas	Flavonoids, steroids, terpenoids, alkaloids, tannins and saponins
Hyptis spicygera	Tannins, sterols, alkaloids, saponin, glycosides and flavonoids
Chromolaena odorata	Flavonoids, saponins, tannins and steroids anthraquinones, alkaloids

Table 4-10: Phytoconstituents of extracts of *T.peruviana*

PLANT EXTRACT	CLASS OF PHYTOCHEMICAL IDENTIFIED	FUNCTIONAL GROUP IDENTIFIED
Petroleum Ether Extract	Terpenoids/ steroids	
Ethanol Extract	General glycosides	Aliphatic aldehyde
Water Extract	General glycosides	Aliphatic aldehyde

Table 4-11: Physical properties and percentage yields of extracts of *T. peruviana*

Extract	Colour	Percentage yield
Petroleum ether	Pale yellow	0.87
Ethanol	Dark brown	6.69
Water	Dark brown	7.85

Table 4-12: Thin layer chromatography results obtained from extracts of *T. peruviana*

Solvent System	Type of Extract	No. of Spots	Rf Values
Butanol: acetronitrile: Water 2:1:1	Ethanol	4	0.113, 0.169, 0.338, 0.437
	Water	3	0.380, 0.024, 0.845
		-	
Butanol: Acetronitrile: Water: 1:1:2	Pet Ether extract	2	0.179, 0627
	Ethanol Extract	3	0.642, 0.702, 0925
	Water extract	4	0.075, 0.702, 0.896, 0.955
Butanol: DMSO: Water: 1:1:2	Pet Ether extract	2	0.134, 0478
	Ethanol Extract	2	0.448, 0567
	Water extract	3	0.149, 0.433, 0.478
Butanol: Acetic Acid : Water 2:2:1	Pet Ether	3	0.077, 0.354, 0.969
	Ethanol Extract	4	0.200, 0.323, 0.615, 0.77
	Water extract	4	0.23, 0.46, 0.723, 0.954
Butanol: Acetronitrile: Water 2:1:1	Pet Ether	-	
	Ethanol Extract	3	0.52, 064, 0.99
	Water extract	3	0.09, 0.77, 093
Butanol: Acetic acid : Water:4:1:5	Pet Ether	-	
	Ethanol Extract	3	0.467, 0.549, 0.958
	Water extract	3	0.127, 0211, 0.409
Butanol: Ethanol: Water:4:1:2:2	Pet Ether	-	
	Ethanol Extract	1	0.33
	Water extract	3	0.141, 0.211, 0.287
Butanol: Acetic acid: Water: 6:4:2	Pet Ether	-	
	Ethanol Extract	2	0.52, 0.78
	Water extract	3	0.22, 0.45, 0.79
Butanol: Acetic Acid: Pet Ether: Water	Pet Ether	1	0.076
	Ethanol Extract	2	0.818, 0939
	Water extract	1	0.803
Butanol: Acetic Acid: Water :2:2:1	Water extract	4	0.21, 0.62, 074, 093
Butanol: Acetic Acid: Water :4:1:1	Water extract	5	0.07, 0.49, 0.60, 0.82, 093
Butanol: Acetronitrile: Water: 1:1:2	Water extract	4	0.10, 0.67, 0.78, 0.94
Chloroform: Methanol (10.1)	Ethanol extract	6	0.02, 0.1, 0.2, 0.3, 0.4, 0.5
Chloroform; Methanol (3:2)	Ethanol extract	2	0.78, 0.96
Chloroform: Methanol (2:3)	Ethanol extract	1	0.98
Pet ether: Water: Chloroform: Methanol	Ethanol extract	3	0.1, 0.2, 0.6

Table 4-10: Thin layer chromatography results of crystals obtained from ethanol extracts

Solvent System	No. of Spots	Rf Values
Pet ether: Water: Ethanol (4:1:1)	-	-
Pet ether: Water: Ethanol (5:1:1)		
	-	-
Trichloromethane: Water: Ethanol (7:1:2)		
	-	-
Trichloromethane: Water: Ethanol(4:1:1)		
	1	1
Trichloromethane: Water: Ethanol(6:1:2)		
	-	-
Trichloromethane: Water: Ethanol(6:2:2)		
	-	-
Butanol: Acetic acid: Water:(2:2:1)		
	1	1
Butanol: Acetic acid: Water: (3:1:1)		
	1	1
Trichloromethane: Water: Ethanol(3:2:1)		
	1	0.6

Table 4-114: Results obtained from the TLC tests on the fractions obtained from the pooled fractions

Pooled Fractions[4]	Colour	Solvent Systems	No. of spots	RF
A (1 -5)	Yellow	$CHCl_3$: Pet ether 6 3	7	0.0.20,0.58 0.68,0.78,0.80 0.94
B (6 - 15)	Pale yellow	$CHCl_3$: Pet ether : EtOH 5 1 1	3	0.50,0.54,0.58
C (16 - 20)	Yellowish brown	$CHCl_3$: Pet ether : EtOH 7 1 1	3	0.45,0.66,0.58
D (21 - 30)	Brown	$CHCl_3$: Pet ether : EtOH	9	0.12,0.26 0.38,0.50 0.58,0.69 0.72,0.79 0.85,

[4] **Pooled fractions obtained from column chromatography**

Table 4-125: Results obtained from functional group tests on *T. peruviana* extracts

SOLVENT SYSTEM USED	GROUP	RESULTS
PET ETHER EXTRACT	Steroids/terpenoids	Positive
	Aldehyde	Negative
	Ketones	Negative
	Esters	Negative
	Carboxylic acid	Negative
	Phenols	Negative
ETHANOL EXPTRACT	Steroids/terpenoids	Negative
	Primary or secondary (Alcohol or aldehyde)	Positive
		Negative
	Ketones	Negative
	Esters	Negative
	Carboxylic acid	Negative
	Phenols	Negative
WATER EXTRACT	Steroids/tepenoids	Negative
	Primary or secondary alcohol or aldehyde	Positive
		Negative
	Ketones	Negative
	Esters	Negative
	Carboxylic acid	Negative
	Phenols	Negative

The phytochemical screening in this study showed that all the experimental plant parts have terpenoids and steroids, and one or more of other phytoconstituents such as general glycosides, tannins, polyphenols, carotenoids, saponins and flavonoids. All these phytochemicals have been reported in earlier works to have antitermitic activities (Trikojus 1935; Nakashima and Shimizu 1972; Chang et al. 2001; Cheng et al. 2004; Ganapaty et al. 2004; Kusumoto et al. 2009).

However, in relating the phytochemicals to the antitermitic activity of the plants one needs to interpret the role of plant chemistry in plant-termite interactions within the framework of three principles; (1) The molecular basis for chemical cues; (2) the molecular diversity of chemical signals which implies specific mechanisms for plant-

initiated attraction or repellence of termites; and (3) that there are dynamic elements to many plant chemical defences (Reichardt 1995). This might explain why the presence of similar phytochemicals notwithstanding, the experimental plants in this study did not have equal antitermitic activity.

Results of phytochemical screening of the extract showed that ethanol extract of *T.peruviana* which was most efficacious in controlling termite infestation, contains glycosides. In the attractancy/repellency tests, the ethanol extract was partitioned in methanol, cyclohexane, chloroform and water. The relatively non-polar fractions cyclohexane and chloroform fractions showed repellency whilst the polar fractions ethanol and methanol fractions showed attrantancy with water fraction showing just borderline repellency. Thus the observed attractancy and toxicity of the ethanol extract of *T.peruviana* may be due to the sugar moiety attached to the poisonous genin and the fact that the genin part may be soluble in non-polar solvents whilst the sugar moiety may be soluble in the polar solvents. The case of water which is expected to dissolve more sugar moiety cannot be explained. However, the attractancy and repellent properties can be employed in the formulation of antitermitic agents.

RESULTS OF ANALYSIS OF COMPOUND A

Compound A was obtained from HPLC analysis of AM1 and was the most predominant peak (APPENDIX 3).

HPLC:
Retention time of A = 6.12 (APPENDIX 3)
MASS SPECTROMETRY: (APPENDIX 4)
Significant peaks are:

763. 7 corresponding to the Molecular formula $C_{40}H_{64}O_{13}$

787.6 corresponding to the Molecular formula $C_{40}H_{64}O_{13}$ Na

291, corresponding to Molecular formula $C_{19}H_{31}O_2$

313, corresponding to Molecular formula $C_{19}H_{31}O_2Na^+$

IR: (APPENDIX 7)

3450cm^{-1} corresponding to O -H

1780cm^{-1} corresponding to α, β, unsaturated γ- lactone

1620cm^{-1} corresponding to C=C

1100cm^{-1} corresponding to C-O

UV $_{max}$: (APPENDIX 8)

220 nm implying α, β, unsaturated γ- lactone with further conjugation

Table 4-136: ^{13}C NMR of compound A (APPENDIX 5)

CARBON NUMBER	ASSIGMENT	CHEMICAL SHIFT
1	Singlet	29.585
2	Singlet	27.863
3	Singlet	49.582
4	Singlet	33.276
5	Singlet	35.931
6	Singlet	26.840
7	Singlet	21.309
8	Singlet	41.760
9	Singlet	35.62- 35.439
10	Singlet	
11	Singlet	21.115
12	Singlet	39.985
13	Singlet	66.768
14	Singlet	85.522
15	Singlet	33.095
16	Singlet	26.425
17	Singlet	50.877
18	Singlet	15.740
19	Singlet	23.680
20	Singlet	174.575
21	Singlet	73.425
22	Singlet	174.575
23	Singlet	117.615
1’	Singlet	95.38
1’’	Singlet	98.19
1’’’	Singlet	98.25
2’	Singlet	34.80
2’’	Singlet	34.75
2’’’	Singlet	32.16
3’	Singlet	30.07
3’’	Singlet	34.75
3’’’	Singlet	32.16
4’	Singlet	30.07
4’’	Singlet	29.69
4’’’	Singlet	26.36
5’	Singlet	21.04
5’’	Singlet	20.55
5’’’	Singlet	18.50
6’	Singlet	17.74
6’’	Singlet	17.74
6’’’		15.77

Table 4-147: ^{1}H NMR of the genin part of compound A ((APPENDIX 6)

CARBON NUMBER	SPLITTING	ASSIGNMENT
1	Triplet of a doublet 1H	1.42
1	Triplet of a doublet1H	1.45
2	Multiplet	1.5
3-OH	Singlet 1H	4.1
3	No signal	
4(Ha)	Multiplet 1H	1.85
4(Hb)	Multiplet 1H	1.32
5	Multiplet 1H	1.7
6(Ha)	Multiplet 1H	1.2
6(Hb)	Multiplet 1H	1.85
7(Ha)	Multiplet 1H	1.2
7(Hb)	Multiplet 1H	1.6
8	Multiplet 1H	1.52
9	Multiplet 1H	1.55
10	No signal	
11(Ha)	Multiplet 1H	1.42
11(Hb)	Multiplet 1H	1.2
12(Ha)	Multiplet 1H	1.49
12(Hb)	Multiplet 1H	1.35
13	No signal	
14	No signal	
14-OH	No signal	
15(Ha)	Multiplet 1H	2.10
15(Hb)	Multiplet 1H	1.6
16(Ha)	Multiplet 1H	1.85
16(Hb)	Multiplet 1H	2.12
17	Doublet of a doublet 1H	2.75
18	Singlet 3H	0.6
19	Singlet 3H	0.7
20	No signal	
21	Quartet 2H	4.85

Table 4-158: ^{1}H NMR of Sugar moiety of compound A (APPENDIX 5)

CARBON NUMBER	SPLITTING PATTERN	ASSIGNMENT
1’	Doublet of a doublet 1H	4.84
2α’	Multiplet of 1H	2.05
2β’	Multiplet of 1H	1.71
3’	Multiplet	4.24
3’-OH	Singlet	3.04
4’	Doublet of a doublet 1H	3.24
5’	Quartet of a doublet	3.78
6’	Doublet of 3H	1.28
1’’	Doublet of a doublet 1H	4.88
2α’’	Quartet Multiplet 1H	2.13
2β’’	QuartetMultiplet 1H	1.71
3’’	Multiplets	4.25
3’- OH	Singlet	2.98
4’’	QuartetDoublet of a doublet 1H	3.21
5’’	Multiplet Quartet of a doublet 1H	3.83
6’’	HDoublet 3H	1.22
1’’’	Doublet of a doublet 1H	4.90
2α’’’	Multiplet !H	2.12
2 β’’’	Multiplet 1H	1.75
3 ’’’	Multiplet	4.13
3’’’-OH	Singlet	2.46
4 -’’’	Doublet of a doublet 1H	3.30
4 ’’’ –OH	Singlet	2.20
5’’’	Quartet of a doublet 1H	3.78
6 ’’’	Doublet of 3H	1.22

The ^{1}H-NMR of the sugar moiety of the compound A showed several splitting patterns as indicated below.

Six doublet of a doublet one-proton at 4.84, 4.88 and 4.90 for 1'-H, 1''-H and 1'''-H respectively and the remaining three at 3.24, 3.21 and 3.30 for 4'-H, 4''-H and 4'''-H respectively and these were attached to carbon 1' , 1'', 1''', 4', 4'' and 4''' at a chemical shift of 95.38, 98.19, 98.25, 26.36, 26.00 and 23.50 respectively. Further splitting gave nine multiplets i.e. (doublet of a doublet of a doublet split by protons of (1', 3' and 2a') for 2b'-H, (1'', 3'' and 2a'') for 2b''-H, (1''', 3''' and 2a''') for 2b'''-H, (1', 3' and 2b') for 2a'-H, (1'', 3'' and 2b'') for 2a''-H, (1''', 3''' and 2b''') for 2a'''-H, and also by (4', 2a' and 2b') for 3'-H, (4'', 2a'' and 2b'') for 3''-H, and (4''', 2a''' and 2b''') for 3'''-H) at a chemical shift value of 145

2.05, 1.71. 4.24 for 2a'-H, 2b'-H and 3' respectively, 2.13, 1.71 and 4.25 for 2a''-H, 2b''-H and 3'' respectively and 2.12, 1.75 and 4.13 for 2a'''-H, 2b'''-H and 3''' respectively. These were also attached to carbons at a chemical shit value of 34.80 and 30.07 for 2' and 3' respectively, 34.75 and 29.61 for 2'' and3'' respectively and 32.16 and 26.36 for 2''' and 3''' respectively.

Three quartets of a doublet one-proton were also showed at 3.78 for 5'-H and 5'''-H and 3.83 for 5''-H, and these were attached to carbons at a chemical shift values of 21.04, 20.55 and 18.50 for 5', 5'' and 5''' respectively. Finally, peaks were also showed at 1.28, 1.22 and 1.22 giving a doublet three-protons attached to carbons at a chemical shift values of 17.74, 17.74 and 15.77 for 6', 6'' and 6''' respectively.

RESULTS OF ANALYSIS OF COMPOUND B

HPLC: (APPENDIX 12)

Retention time : 4.91min

Melting point of B: 255-257

Table 4-169: ^{1}HNMR assignment of compound B (APPENDIX 14)

CARBON NUMBER	SPLITTING PARTERN	CHEMICAL SHIFT
1	Triplet 1H	1.43
1	Triplet 1H	1.44
2	Triplet of a doublet 2H	1.6
3	Multiplet 1H	4.15
4(Ha)	Multiplet 1H	1.8
4(Hb)	Multiplet 1H	1.3
5	Multiplet 1H	1.7
6(Ha)	Multiplet 1H	1.2
6(Hb)	Multiplet 1H	1.85
7(Ha)	Multiplet 1H	1.2
7(Hb)	Multiplet 1H	1.6
8	Multiplet 1H	1.52
9	Multiplet 1H	1.55
10	No signal	
11(Ha)	Multiplet 1H	1.42
11(Hb)	Multiplet 1H	1.2
12(Ha)	Multiplet 1H	1.49
12(Hb)	Multiplet 1H	1.35
13	No signal	
14	No signal	
15(Ha)	Multiplet 1H	2.10
15(Hb)	Multiplet 1H	1.6
16(Ha)	Multiplet 1H	1.85
16(Hb)	Multiplet 1H	2.12
17	Doublet of a doublet 1H	2.75
18	Singlet 3H	0.8
19	Singlet 3H	0.9
20	No signal	
21	Quartet 2H	4.85
22	No signal	
23	Singlet 1H	5.8

Table 4-20: ^{13}C NMR assignment of Compound B (APPENDIX 13)

CARBON NUMBER	SPLITTING PARTERN	CHEMICAL SHIFT(^{13}C-nmr)
1	Singlet	29.62
2	Singlet	27.88
3	Singlet	49.62
4	Singlet	33.29
5	Singlet	35.96
6	Singlet	26.87
7	Singlet	21.35
8	Singlet	41.79
9	Doublet	35.39-35.47
10	Singlet	
11	Singlet	21.15
12	Singlet	40.02
13	Singlet	66.79
14	Singlet	85.56
15	Singlet	33.13
16	Singlet	26.46
17	Singlet	50.91
18	Singlet	15.77
19	Singlet	23.72
20	Singlet	174.65
21	Singlet	73.47
22	Singlet	174.57
23	Singlet	117.65

RESULTS OF ANALYSIS OF COMPOUND C

Melting point: 185-188^{o}C

Mass Spectroscopy: major peaks are; ((APPENDIX 16)

160 corresponding to the Molecular formula $C_6H_{12}O_4$

178 corresponding to the Molecular formula $C_6H_{11}O_3Na+$

341.3 corresponding to the Molecular ion with formula $C_{12}H_{22}O_{11}$

365.2 corresponding to the Molecular formula $C_{12}H_{21}O_{11}Na+$

387.3 corresponding to the Molecular formula $C_{13}H_{23}O_{13}$

IR:

3450cm^{-1} corresponding to OH

-- 1050cm^{-1} corresponding to C-O

UV_{max} : 299 (APPENDIX 17)

POLARIMETRY:

Plane polarized light was rotated clockwise implying Compound C is dextrorotatory.

RESULTS OF ANALYSIS OF COMPOUND D

GC-MS: Four strong peaks (Fig. 4-12 to 4-16)
Retention time

- Component 1 is 16.92
- Component 2 is 16.60
- Component 3 is 14.67
- Component 4 is 13.92

Component 1 and 2 are enanantiomers and were resolved because a

Major peaks in their various spectra are

41 corresponding to the Molecular formula C_3H_5

124 corresponding to the Molecular formula $C_7H_8O_2$

162 corresponding to the molecular formula $C_{10}H_{10}O_2$

175 corresponding to the Molecular formula $C_{11}H_{11}O_2$

203 corresponding to the Molecular formula $C_{12}H_{11}O_3$

247 corresponding to the Molecular formula $C_{13}H_{11}O_5$

374 corresponding to the Molecular ion peak $C_{23}H_{41}O_4$

Table 4-21: ^{1}HNMR assignment Compound D (see page Fig. 4-20 to 4-24)

CARBON NUMBER	SPLITTING PARTERN	CHEMICAL SHIFT
1	Triplet 1H	1.42
1	Triplet 1H	1.45
2	Triplet of a doublet 2H	1.5
3	Multiplet 1H	4.1
4(Ha)	Multiplet 1H	1.85
4(Hb)	Multiplet 1H	1.32
5	Multiplet 1H	1.7
6(Ha)	Multiplet 1H	1.2
6(Hb)	Multiplet 1H	1.85
7(Ha)	Multiplet 1H	1.2
7(Hb)	Multiplet 1H	1.6
8	Multiplet 1H	1.52
9	Multiplet 1H	1.55
10	No signal	
11(Ha)	Multiplet 1H	1.42
11(Hb)	Multiplet 1H	1.2
12(Ha)	Multiplet 1H	1.49
12(Hb)	Multiplet 1H	1.35
13	No signal	
14	No signal	
15(Ha)	Multiplet 1H	2.10
15(Hb)	Multiplet 1H	1.6
16(Ha)	Multiplet 1H	1.85
16(Hb)	Multiplet 1H	2.12
17	Doublet of a doublet 1H	2.75
18	Singlet 3H	0.6
19	Singlet 3H	0.7
20	No signal	
21	Quartet 2H	4.85
22	No signal	
23	Singlet 1H	5.85

Table 4-22: ^{13}C NMR assignment of compound D (See Fig. 4-20 to 4-24)

CARBON NUMBER	SPLITTING PARTERN	CHEMICAL SHIFT(^{13}C-nmr)
1	Singlet	29.585
2	Singlet	27.863
3	Singlet	49.582
4	Singlet	33.276
5	Singlet	35.931
6	Singlet	26.840
7	Singlet	21.309
8	Singlet	41.760
9	Doublet	35.62- 35.439
10	Singlet	
11	Singlet	21.115
12	Singlet	39.985
13	Singlet	66.768
14	Singlet	85.522
15	Singlet	33.095
16	Singlet	26.425
17	Singlet	50.877
18	Singlet	15.740
19	Singlet	23.680
20	Singlet	174.575
21	Singlet	73.425
22	Singlet	174.575
23	Singlet	117.615

The ^{1}H-NMR of compound D showed splitting patterns as described below.

Eighteen different multiplets one-proton i.e. (doublet of a doublet of a doublet splitted by protons of 1a, 1b and 3) for 2-H at a chemical shift of 1.5 attached to a carbon with a chemical shift value of 27.863, (doublet of a doublet of a doublet splitted by protons of 4a,

3 and 5) for 4b-H and (by protons of 4b, 3 and 5) for 4a-H at 1.32 and 1.85 respectively and were attached to C-4 at 33.276 (doublet of a doublet of a doublet of a doublet splitted by protons of 4a, 4b, 6a, and 6b) for 5-H at 1.7 attached to a C-5 at 35.931, (doublet of a doublet of a doublet of a doublet splitted by protons of 7a, 7b, 6a, and 5) for 6b at 1.85 and 1.2 respectively and these were attached to C-6 at 26.840, (doublet of a doublet of a doublet of a doublet splitted by protons of 6a, 6b,7b and 8) for 7a-H and (by protons of 6a, 6b, and 8) for 7b-H at 1.2 and 1.6 respectively and these were attached to C-7 at 21.309, (doublet of a doublet of a doublet splitted by protons of 7a, 7b and 9) for 8-H at 1.52 attached to C-8 with a chemical shift value of 41.760, (doublet of a doublet of a doublet splitted by protons of 11a, 11b and 8) for 9-H at 1.55 attached to C-9 at 35.362-35.439, (doublet of a doublet of a doublet splitted by protons of 11b, 12a and 12b) for 11b-H at 1.42 and 1.2 respectively and theses were attached to C-11 at 21.115, (doublet of a doublet of a doublet splitted by protons of 11a, 11b and 12a) for 12b-H at 1.49 and 1.35 respectively and were attached to a C-12 with a chemical shift value of 39.985, (doublet of a doublet of a doublet splitted by protons of 15b, 16a and 16b) for 15a-H and (by protons of 15a, 16a and 16b) for 15b-H at 2.10 and 1.6 respectively and were attached to C-15 at 33.095, (doublet of a doublet of a doublet of a doublet splitted by protons of 17, 15a, 15b and 16b) for 16a-H and (by protons of 17, 15a, 15b and 16a) for 16b-H at 1.85 and 2.12 respectively and these were attached to C-16 at 26.425. Two triplet of a doublet one-proton were also showed at 1.142, 1.45 and 2.75 for 1b-H, 1a-H and 17-H respectively, and these were connected to C-1and C-17 at 29.585 and 50.877 respectively. Further splitting also gave two singlet one-proton at 4.1 for the hydroxyl proton of C-3 and 5.85 for 23-H, and two other singlets three-protons at 0.6 for 18-H and 0.7 for 19-H, and

these were attached to carbons at 49.582, 117.615, 15.740 and 23.680 for C-3, C-23, C-18 and C-19 respectively. Finally one broad quartet two-protons gave a peak at 4.85 and was attached to C-21 at 73.425.

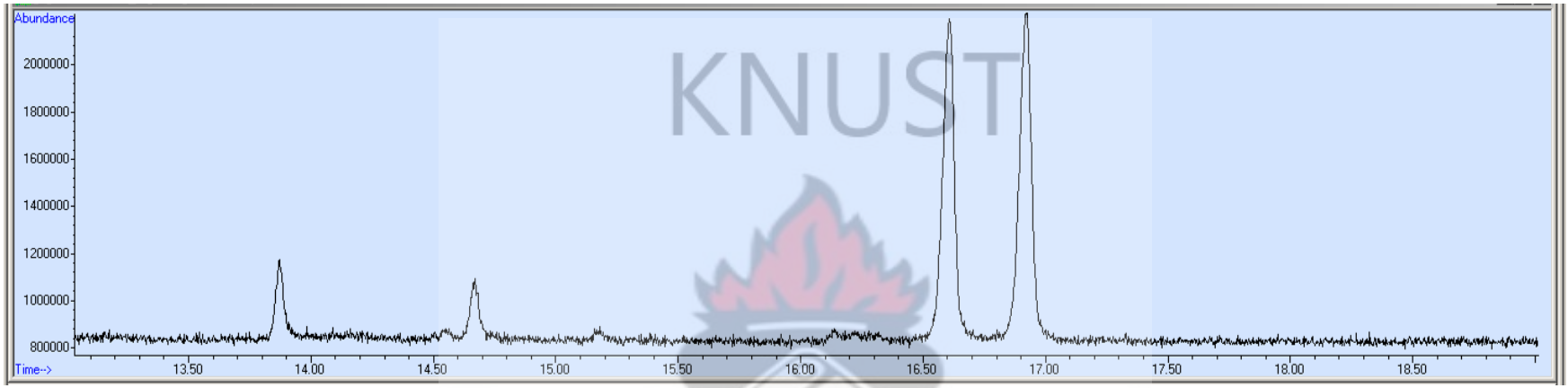


Figure 4-12: Gas chromatogram of compound D

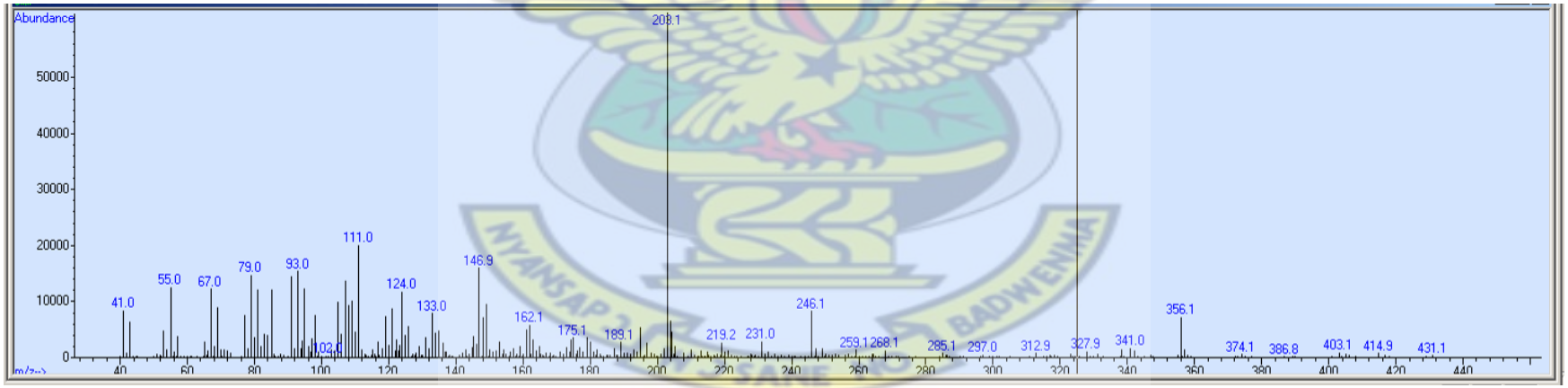


Figure 4-13: Mass spectrum of component with retention time 16.92 minutes

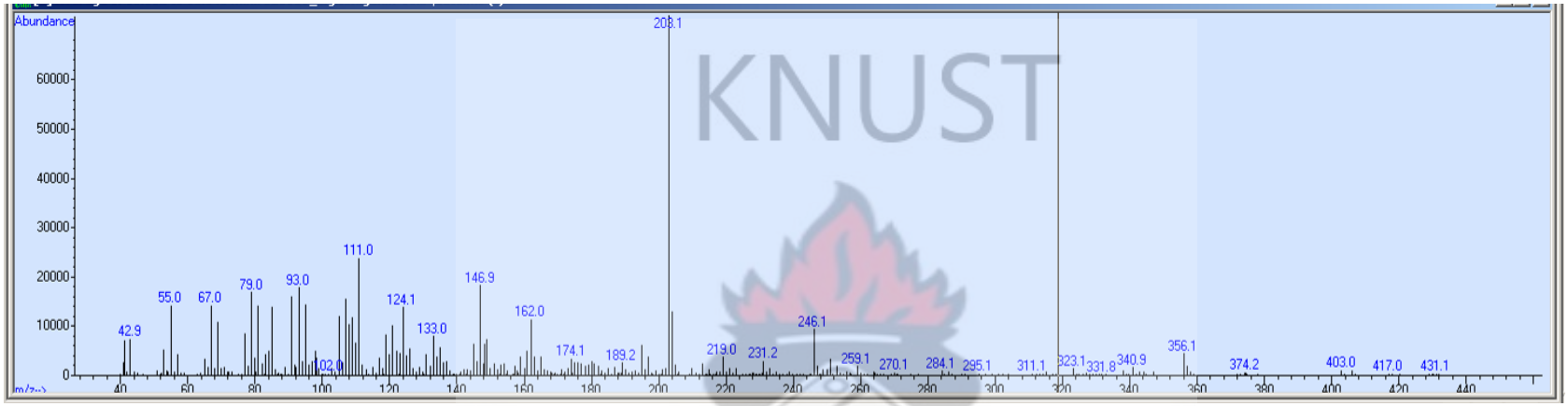


Figure 4-14: Mass spectrum of component with retention time 16.60 minutes

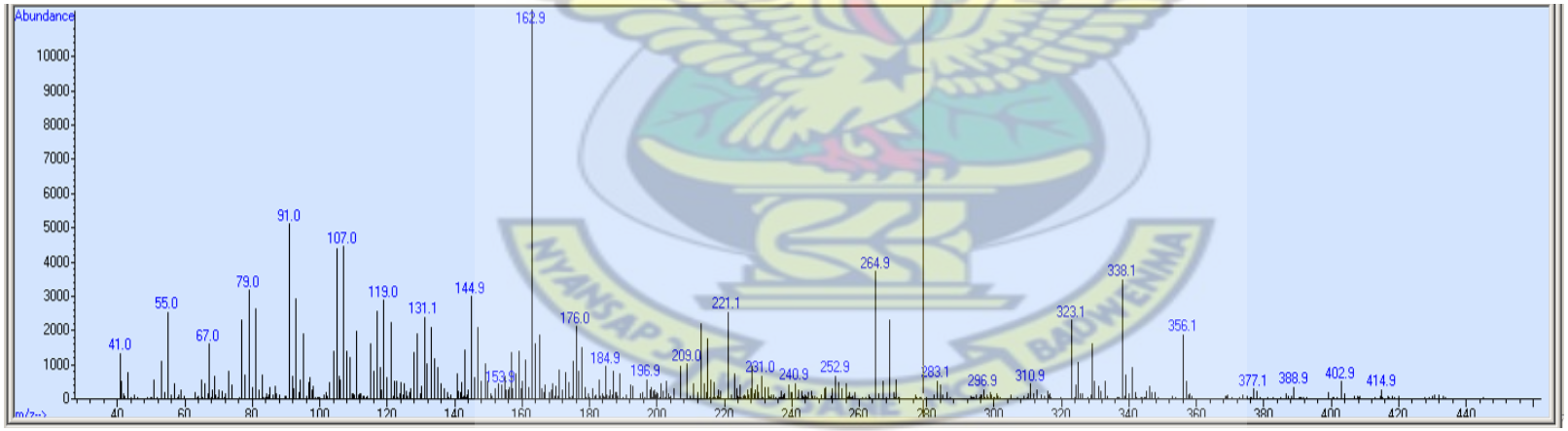


Figure 4-15: Mass spectrum of component with retention time 14.67 minutes

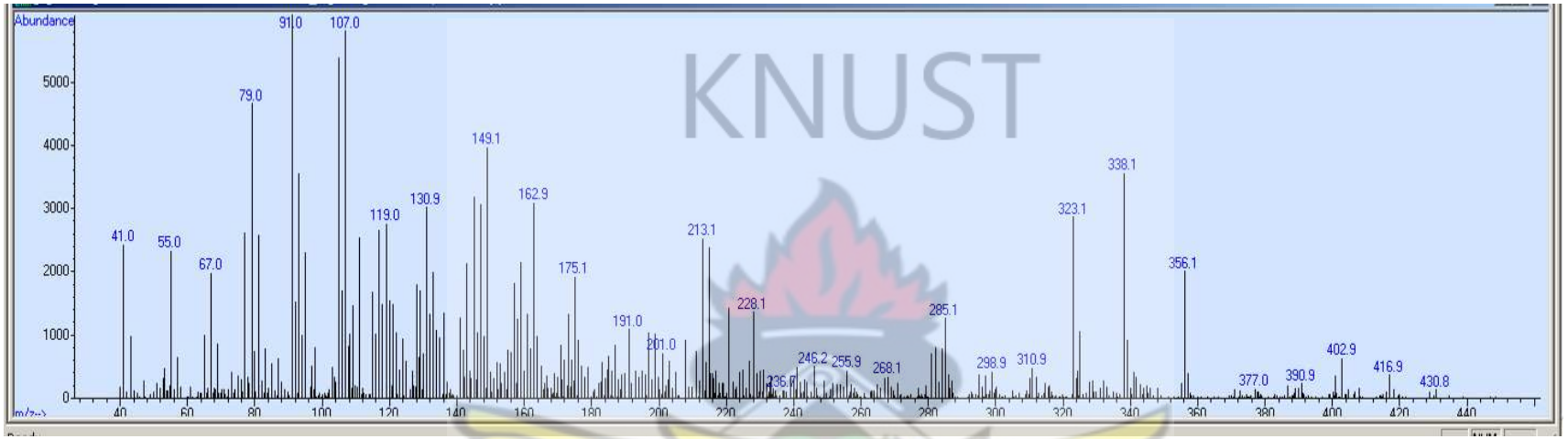


Figure 4-16: Mass spectrum of component with retention time 13.92 minutes

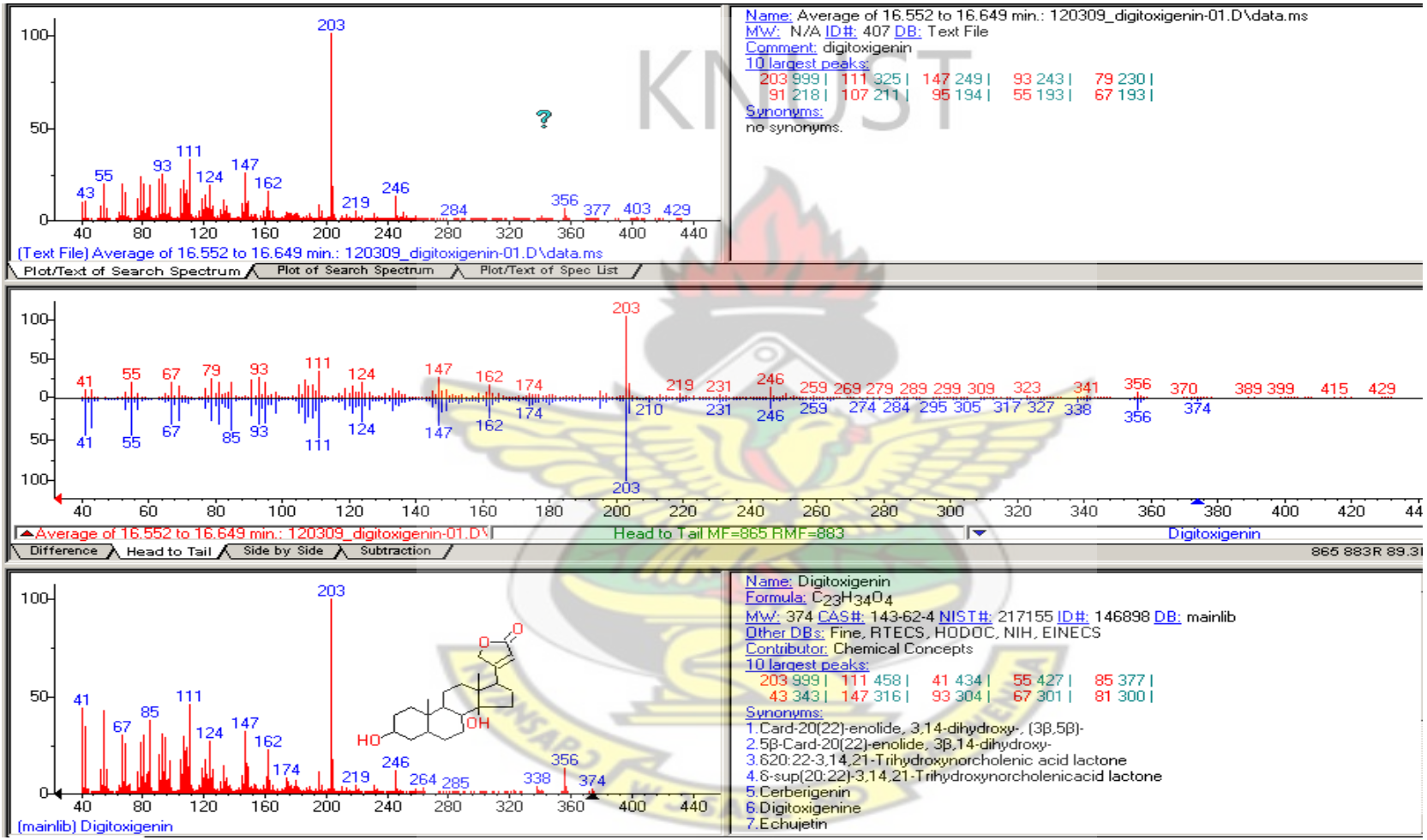


Figure 4-17:Library search of mass spectrum result of component with retention time 19.92

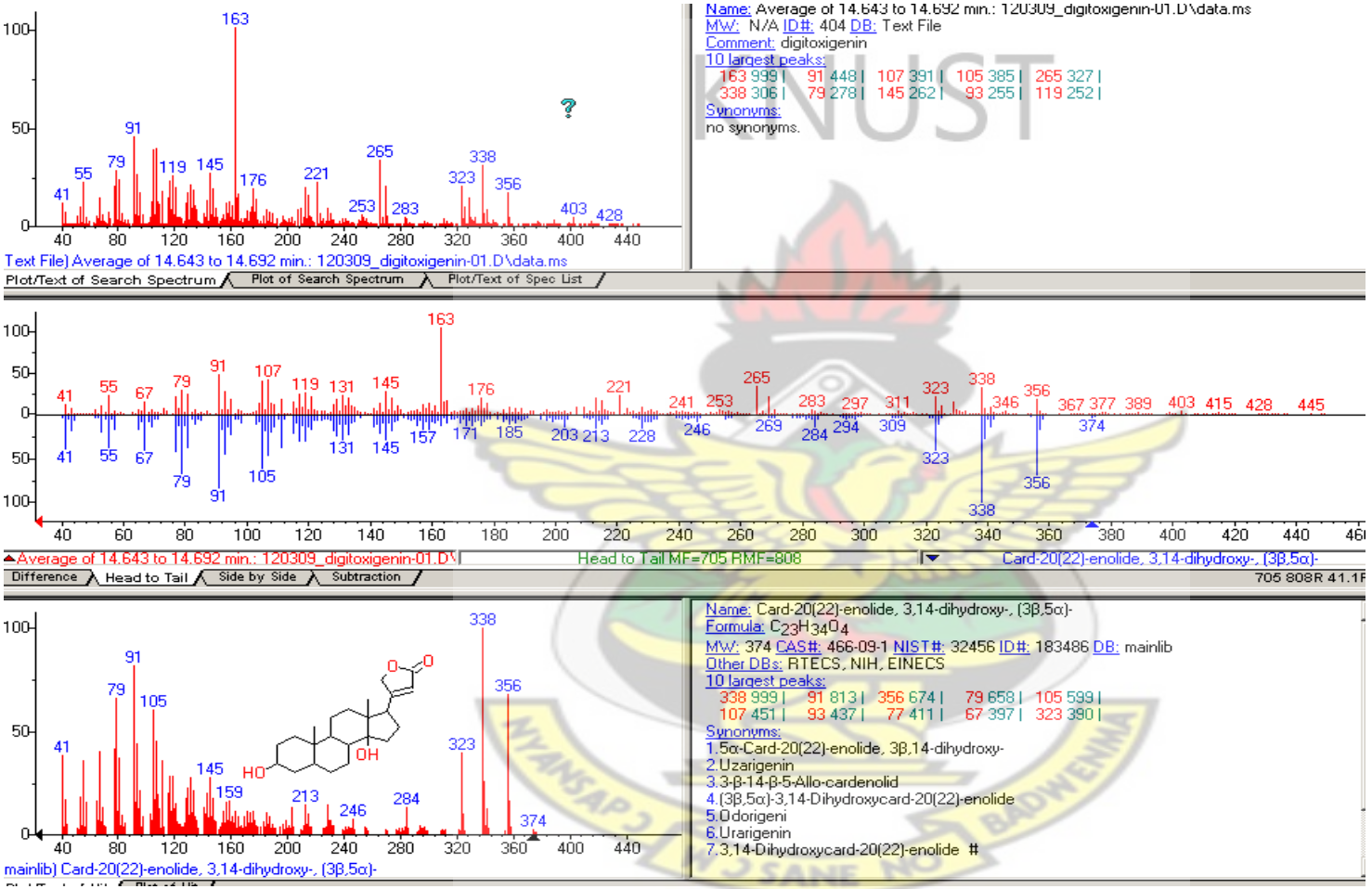


Figure 4-18: Library search of mass spectrum result of component with retention time 19.92

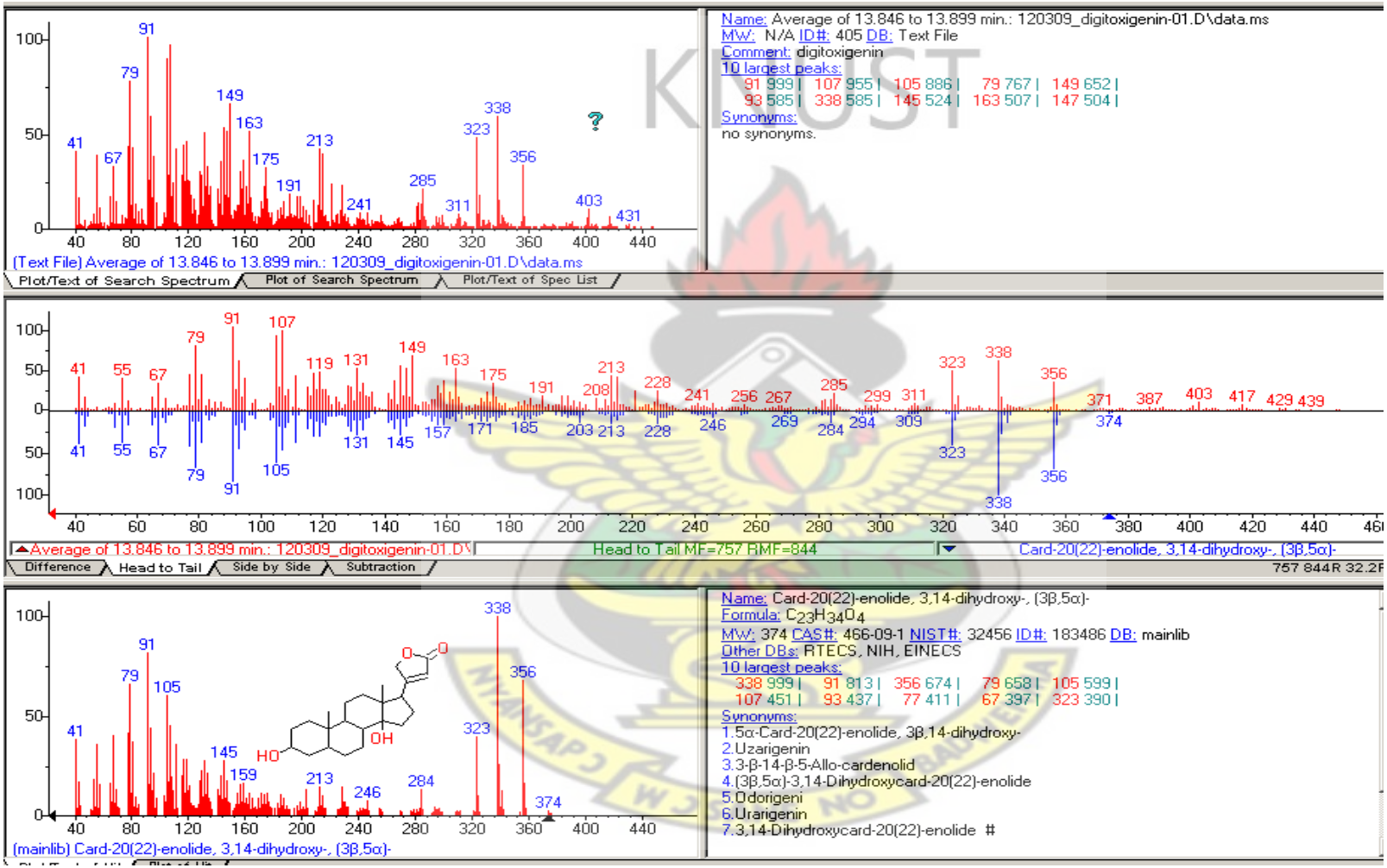


Figure 4-19: Library search of mass spectrum result of component with retention time 19.92

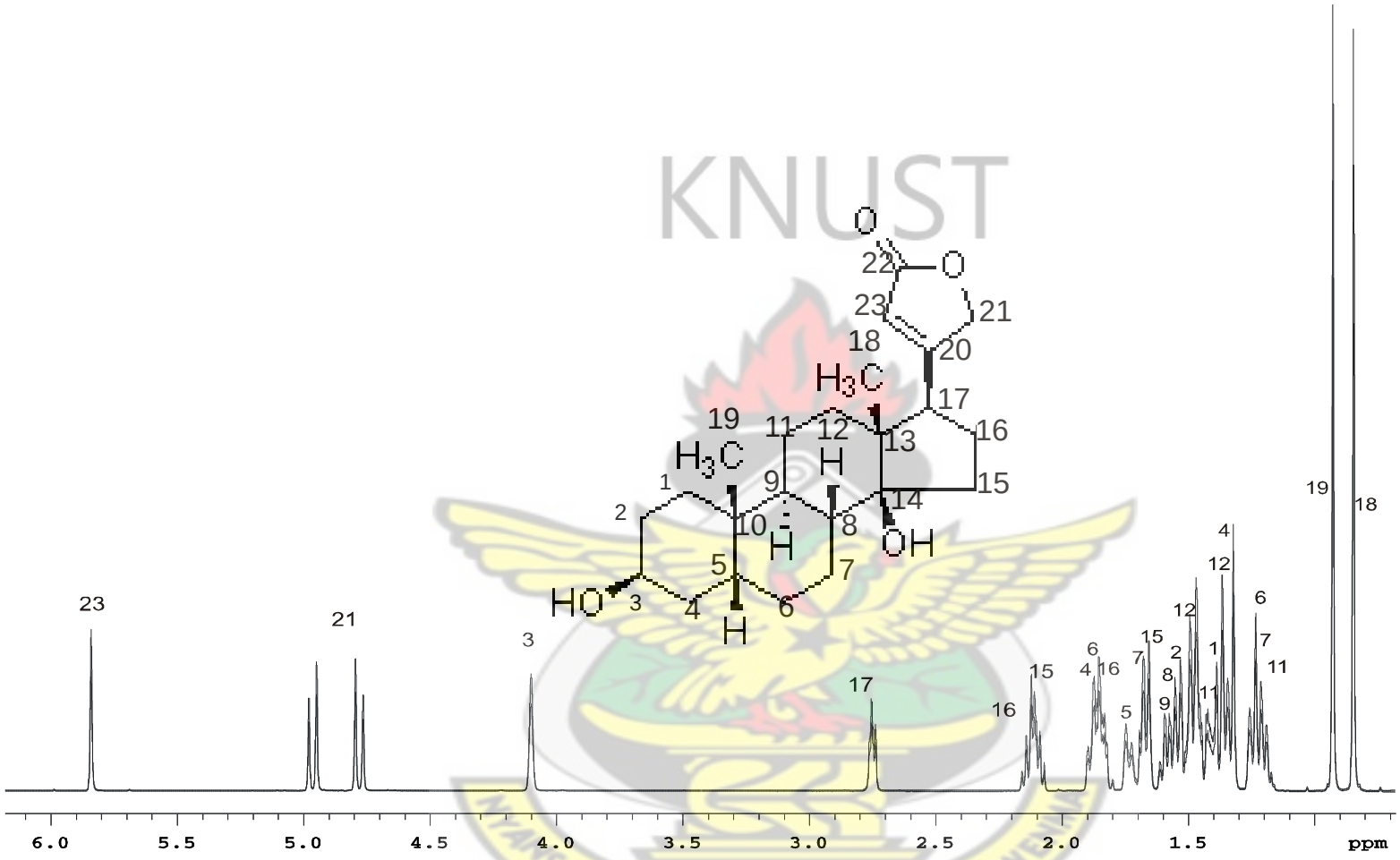


Figure 4-20: H1 spectrum

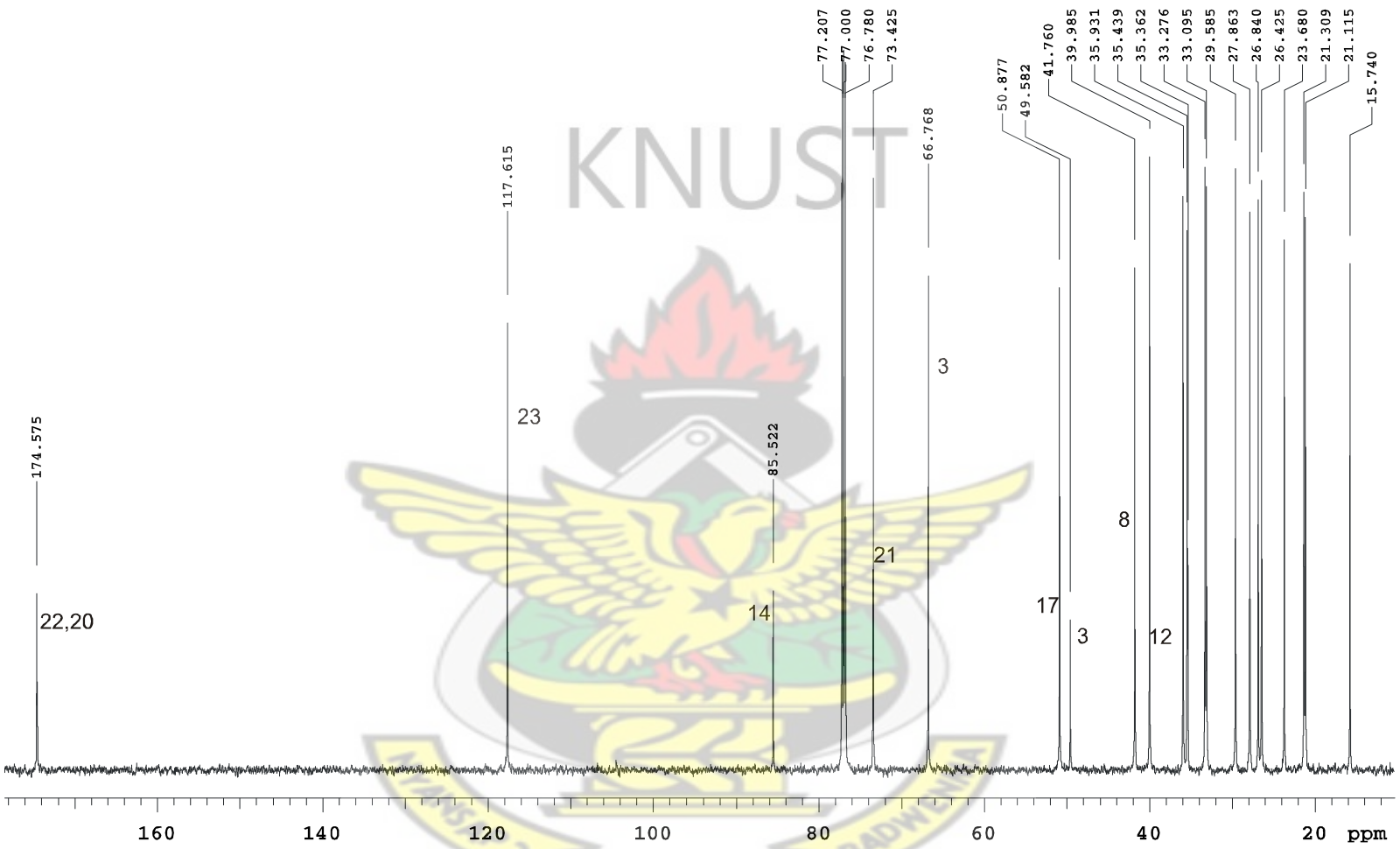


Figure 4-21: C13 spectrum

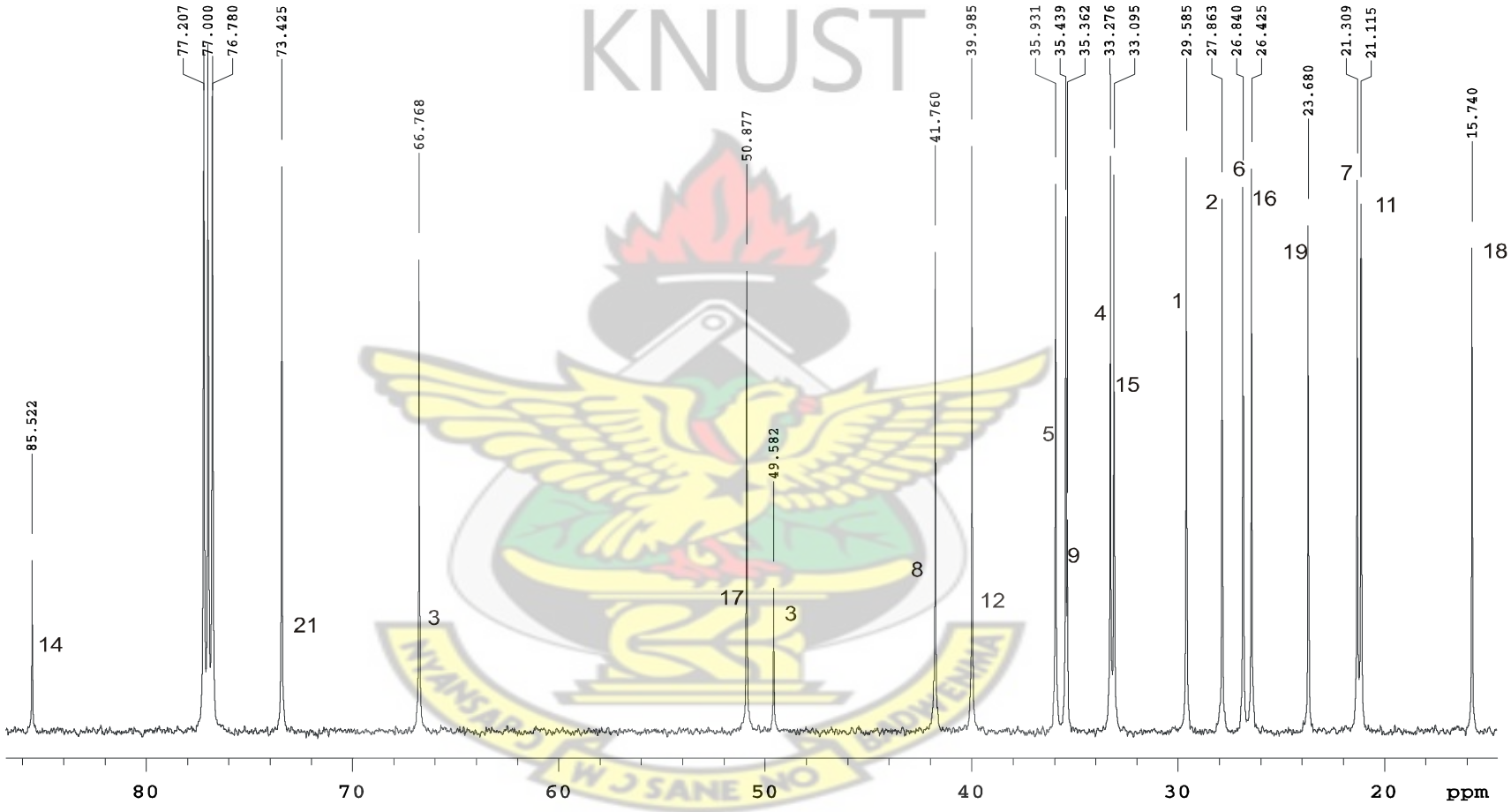


Figure 4-22: Expanded C13 spectrum

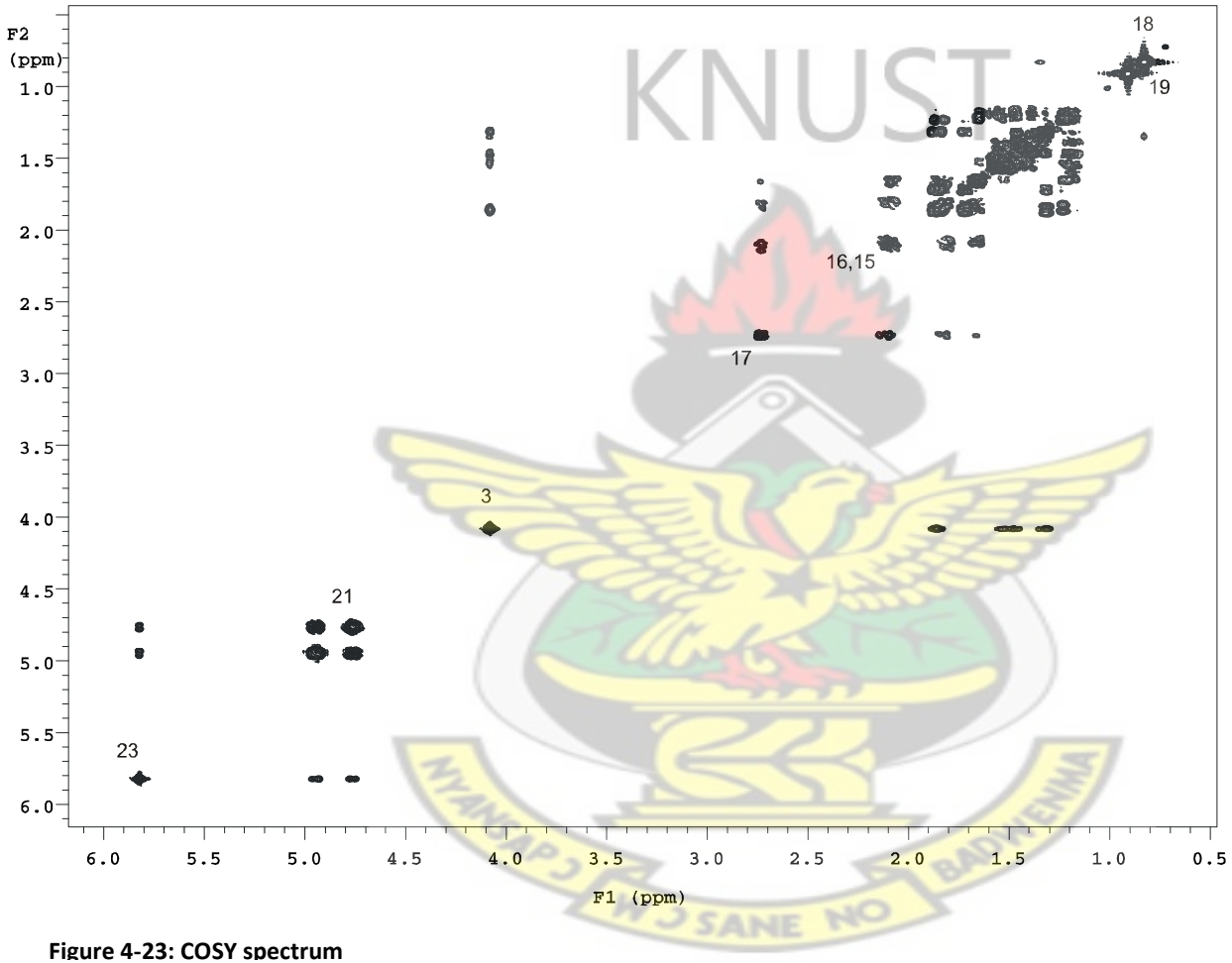


Figure 4-23: COSY spectrum

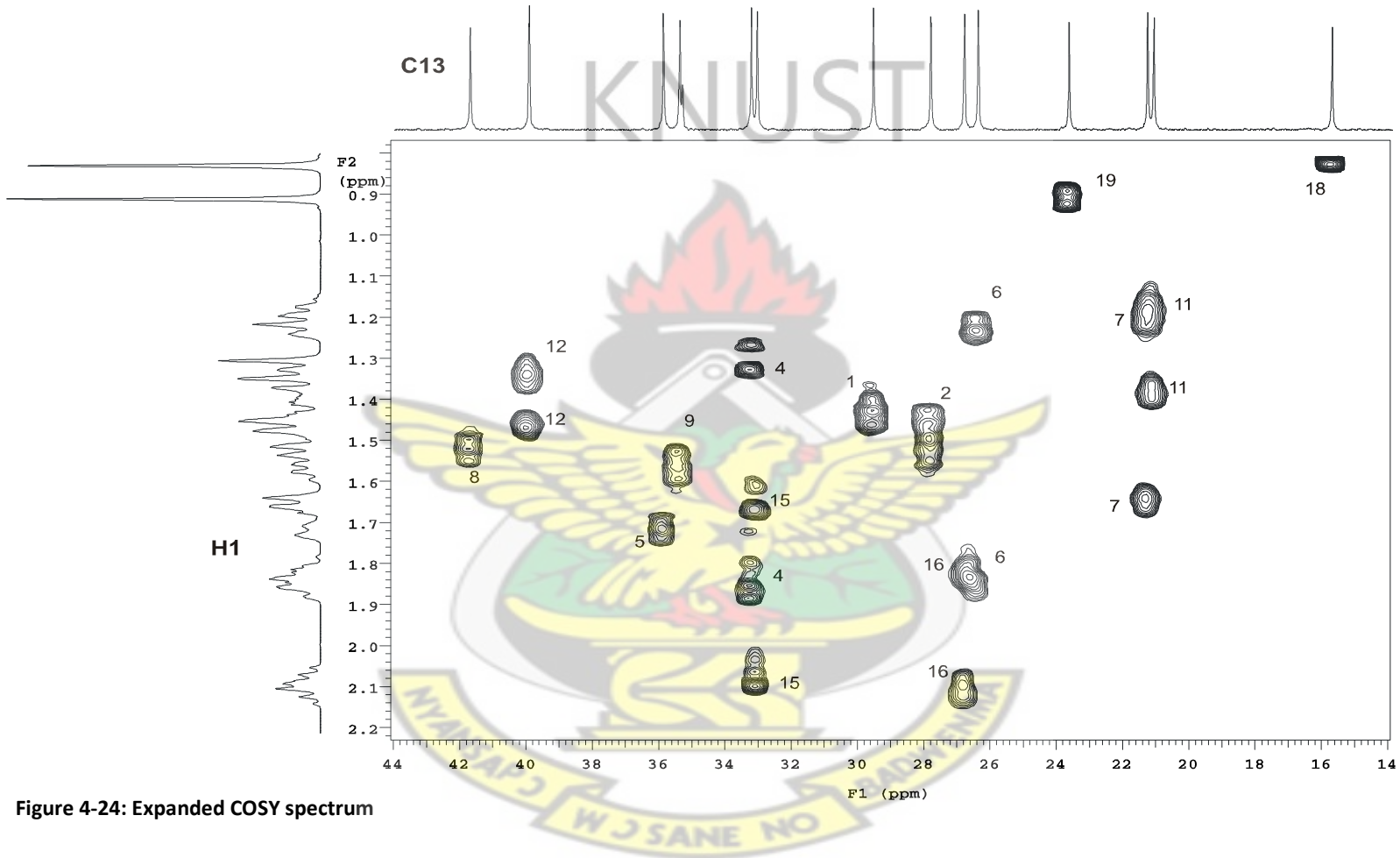


Figure 4-24: Expanded COSY spectrum

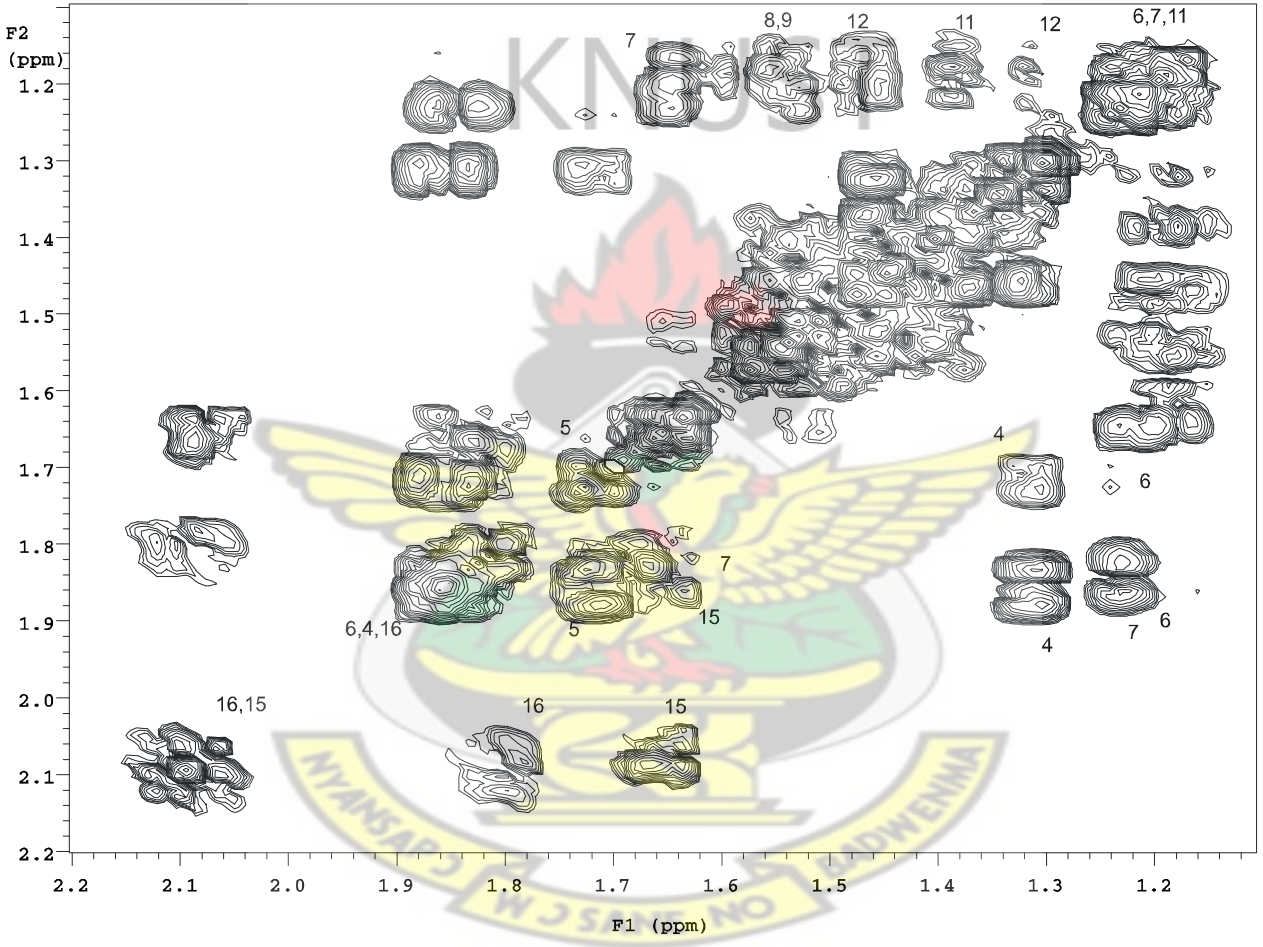
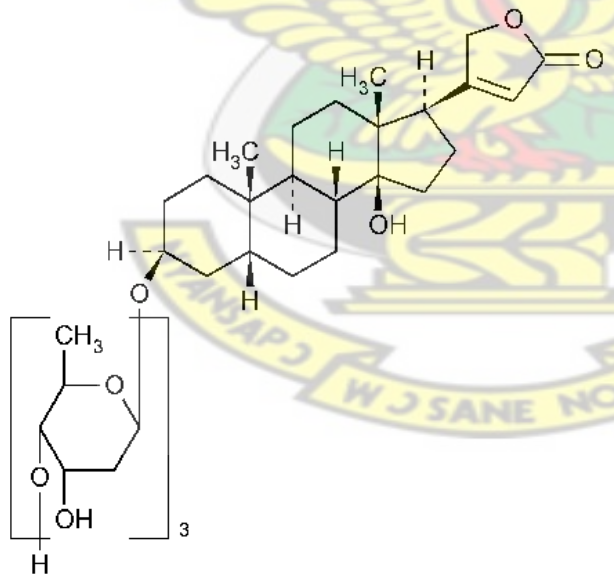


Figure 4-25: Expanded HMQC spectrum. Overlapped proton resonances are resolved by the C13 – H1 correlations.

4.3 IDENTITY OF ACTIVE CONSTITUENTS

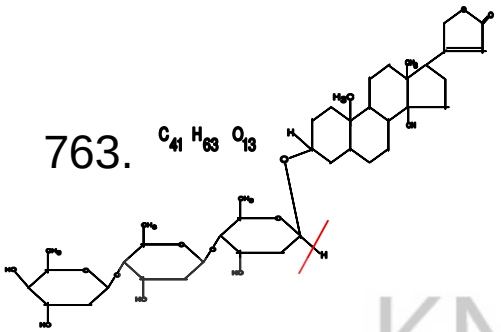
The melting point of compound A was determined to range between 229 and 233^{o}C and its positive ES indicated an ion at m/z = 763.7 (Jakovljevic 1974; Langenhan et al. 2008). This is in agreement with the molecular formula $C_{41}H_{64}O_{13}$. The UV spectrum of compound A showed a maximum at 220nm which indicated the presence of an α and β - unsaturated γ - lactones with further conjugation while the IR spectrum showed bands at 3450 (OH), 1780, 1740 (α, β – unsaturated γ- lactone) 1620 (C=C) cm $^{-1}$ and 1100 (C-O)/ cm.

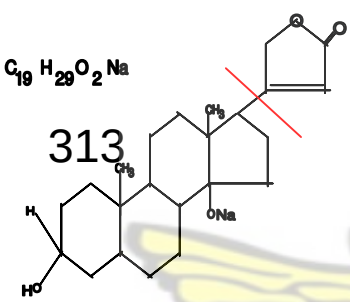
The ^{1}HNMR and ^{13}CNMR assignment in Tables 4-16, 4-17 and 4-18 for compound A were generally in agreement with those previously reported for digitoxin with small differences in chemical shifts arising from the temperature dependence of the chemical shifts. Compound A is therefore digitoxin and its structure is shown below:

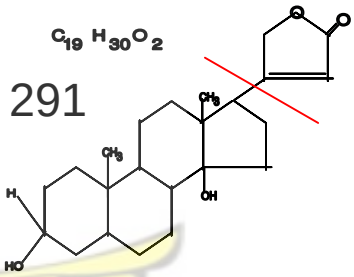


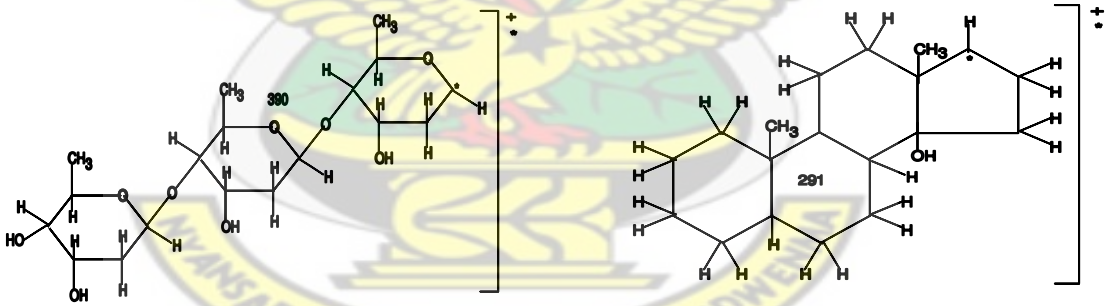
Card-20(22)-enolide,3-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy,(3β,5β)-.

Some probable fragmentation patterns of Compound A are shown below:



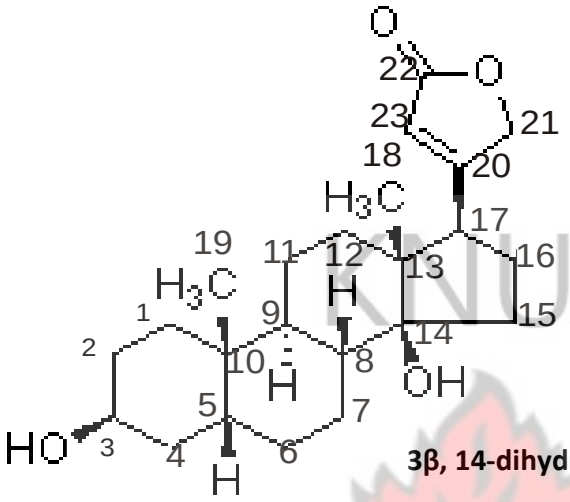






The melting point of compound B was determined to range between 253 and 255^{O}C; 254^{O}C from literature (Gobbini et al. 1998; Jensen et al. 2011). The mass spectra was similar to those previously obtained and reported for digitoxigenin (Appendix).The ^{I}H NMR and ^{13}CNMR assignment in Tables 4-19 and 4-20 for compound B were generally in agreement with those previously reported for Digitoxigenin with small differences in

chemical shifts arising from the temperature dependence of the chemical shifts. Compound B is therefore digitoxigenin and its structure is shown below:



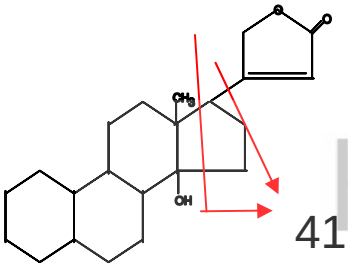
3β, 14-dihydroxy-5β, 14β-card-20(22)-enolide

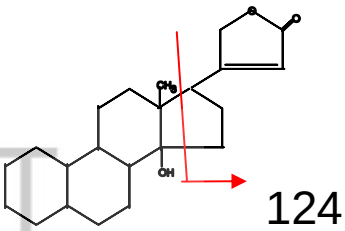
4.4 DERIVATIVE

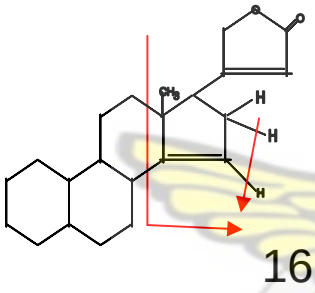
The melting point of compound D was determined to range between 254 and 256^{O}C; 254^{o}C from literature (Gobbini et al. 1998; Jensen et al. 2011) and its GC-MS spectra indicated four well resolved peaks and an ion at m/z 374 which is in agreement with the molecular formula of $C_{23}H_{34}O_{4.}$ The mass spectra was similar to those previously obtained and reported for digitoxigenin (Appendix)

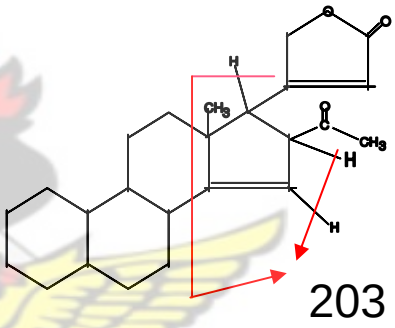
The ^{l}H NMR and ^{13}CNMR assignment in Tables 4-19 and 4-20 for compound D were generally in agreement with those previously reported for Digitoxigenin with small differences in chemical shifts arising from the temperature dependence of the chemical shifts. Compound D is therefore the same as digitoxigenin.

Some possible fragmentation patterns for Compound D are shown below





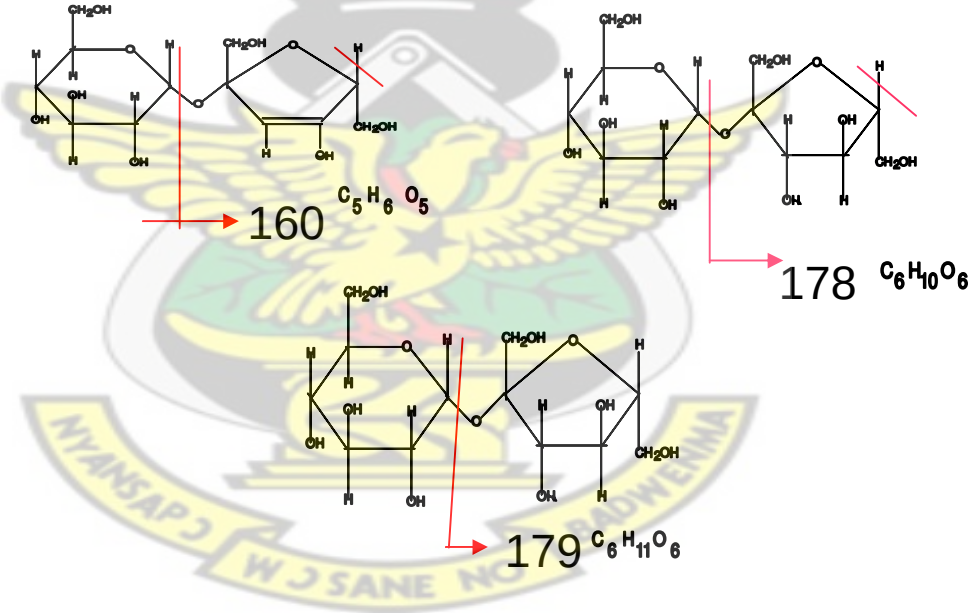




4.5 OTHER CONSTITUENTS

The melting point ofcompound C was determined to be between 185 and 188^{O}C (Mathlouthi et al. 1986; Beckett et al. 2006). The negative ES of the compound indicated an ion at m/z = 341.2 corresponding to the formula $C_{12}H_{21}O_{11}$ and positive ES indicated an ion at m/z =365.2 corresponding to the adduct $C_{12}H_{21}O_{11}Na^{+}$. Its IR spectrum showed bands at 3450 (OH)cm^{-1} and 1050 C-O(cm^{-1}). C was optically active and turned plane polarized light in clockwise direction i.e. dextrorotary (Mathlouthi et al. 1986; Beckett et al. 2006). C was therefore identified as Sucrose and the structure is shown below:

Some fragmentation patterns for compound C is shown below:



Phytoconstituents have been shown to mediate or influence plant-termite interactions and form the molecular basis for the signals or cues that termites use in making dietary choices (Reinhard and Kaib 2001; Evans et al. 2005; Inta et al. 2009). There is no general structural feature or functional group which characterizes deterrents or attractants.

Rather, it seems that molecules from a variety of structural classes play key roles in plant/termite interactions (Salminen 2003; Ngono Ngane et al. 2006; Ross et al. 2007). Even in polymeric materials, minor structural differences can impart very different biological properties (Salminen 2003; Ngono Ngane et al. 2006; Ross et al. 2007). Thus there may be differences in the molecules of glycosides in ethanol extracts of *T. peruviana* and that of water extracts hence the observed differences in repellency/attractancy.

The molecular diversity observed in chemicals that mediate plant-animal interactions implies the existence of specific mechanisms by which they act. For example, while bromides like tannins are feeding deterrents due to their astringency, phenol glycosides render plants unpalatable because they have a bitter taste. A deterrent substance must be detectable by termites, and it has been shown that molecular structure plays an important role in the ways in which termites perceive secondary chemicals (Frazier 1992). However, for a perceived substance to act as a deterrent, its occurrence must be coupled with some deleterious factor. Obviously, the best deterrent would be one which incorporates both signalling and deleterious properties. The plant-derived feeding deterrents must have properties which allow them to be uniquely perceived by termites and have at least the potential for adversely affecting the termite (Parker et al. 2006).

There is a dynamic element to many plant defences, even some which traditionally have been considered to be static (Tripathi and Sharma 2006; Stow and Beattie 2008). The most obvious examples of dynamic plant defences are those classified as induced defences in which a plant's response to damage is either de novo synthesis of defensive substances (Swain et al. 2009) or increased synthesis and storage of toxins (Verma and Singh 2006). In

some cases, the concentrations of defensive chemicals in juvenile plants are significantly higher than those in mature plants. In these cases, at least the level of defence diminishes as an individual plant matures, and the level of defence provided by these deterrents for a given species can greatly increase throughout an entire ecosystem if the plants respond to intense browsing by a juvenile reversion (Deka et al. 2002; Prusak et al. 2005; Mburu et al. 2007).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

Thevetia peruviana has been used as an abortifacient, to treat congestive heart failure, malaria, leprosy, indigestion, ringworm, venereal disease and even as a suicide instrument. Indeed as indicated by the results of this study its list of many uses may now be extended to include natural antitermitics. This work has shown that the potential for the use of anti-termitic agents from plants is promising and that these agents maybe used in three main ways; i.e. using the crude antitermitic agent or extracted in a suitable solvent, as purified product and as a lead compound in synthesizing novel antitermitic agents. The powdered root and ethanol extract of *T. peruviana* may have three beneficial uses: as termite barrier under people's houses; as a method of killing termites directly when there are incursions: and to impregnate and protect wood used in new housing or other wood construction. Ethanol extract of *T. peruviana* could be formulated into a bait or the pulverized root may be considered as possible repellent mulch which may serve as an additional barrier for household and farm level protection against termites. General glycosides found in *T.peruviana* may be useful as natural termite repellent agent and the structures identified could be used as lead compounds for the development of termite repelling and other agents for the protection of crops, trees and other wood products termite against termites' damage.

T. peruviana is a common plant that grows freely in most communities and this finding is of great economic significance especially in Ghana and other tropical countries where individuals mostly affected are poor and unable to afford expensive imported synthetic termiticides for the protection of their properties. In addition, the majority of these

individuals are illiterate and may not readily grasp the technicalities involved in the application of termiticides and may expose themselves to health hazards.

The Building and Road Research Institute of the Council for Scientific and Industrial Research Institute could embark on pilot production trials and testing of anti -termite agents from *T. peruviana* with the purpose of using them as local alternatives in the control of termites.

Since *T. peruviana* can thrive very well in degraded soils, large scale cultivation of this plant and other potential anti-termite plants should be encouraged and this would serve as additional sources of termite controlling agents.

The Building and Road Research Institute is involved in winning of clay for its bricks, tiles and pozzolana factories *T. peruviana* and other plants with potential anti-termite properties could be planted on these sites to help in reclamation of these sites and these plants could be used for pilot production and testing of anti-termite control agents. Other companies involved in winning of clays and sand could also be encouraged to adopt this practice.

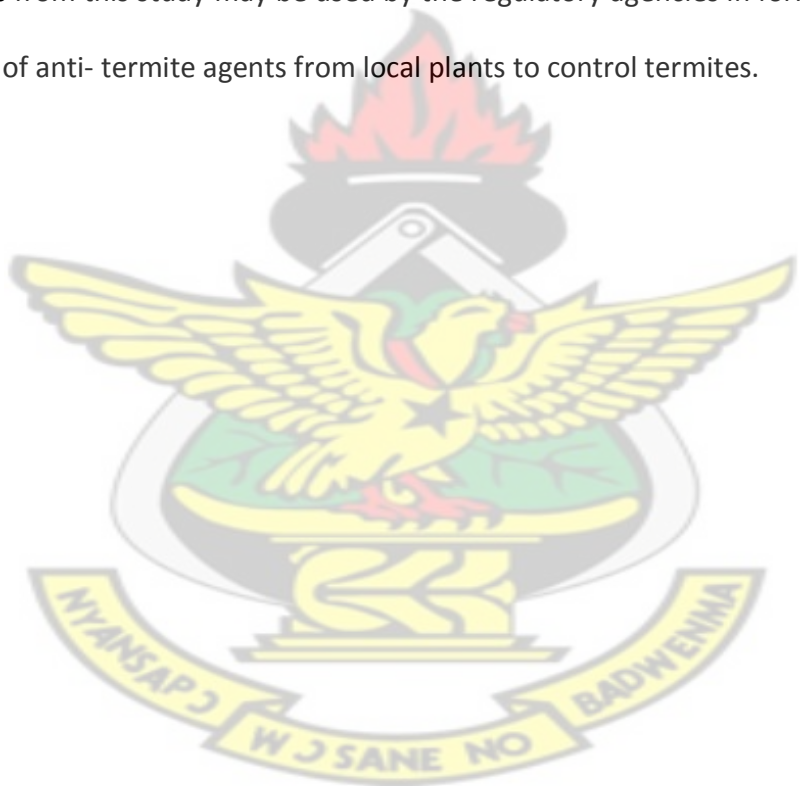
Studies into the termite controlling activities of *T-peruviana* in nurseries of crops and trees plantations could be carried out by scientists in the Building and Road Research Institute, Crops Research Institute and Forestry Research Institute all of the CSIR to determine applications that can be integrated into and augmented with existing termite management programmes.

Training programs could be organized for the public on the use of simple formulations of *T.peruviana* to control termite infestation. This would be particularly useful in the rural

areas where most buildings are of mud and are highly infested with termites and where majority of the inhabitants are resource limited farmers who cannot afford commercial insecticides. Awareness program on the dangers posed by termites and how they can be controlled especially with anti-termite control agents from plants should be intensified by the Building and Road Research Institute of the CSIR.

There is the need for the Building and Road Research Institute to replicate this study in other parts of the country.

Findings from this study may be used by the regulatory agencies in formulating policies on the use of anti- termite agents from local plants to control termites.



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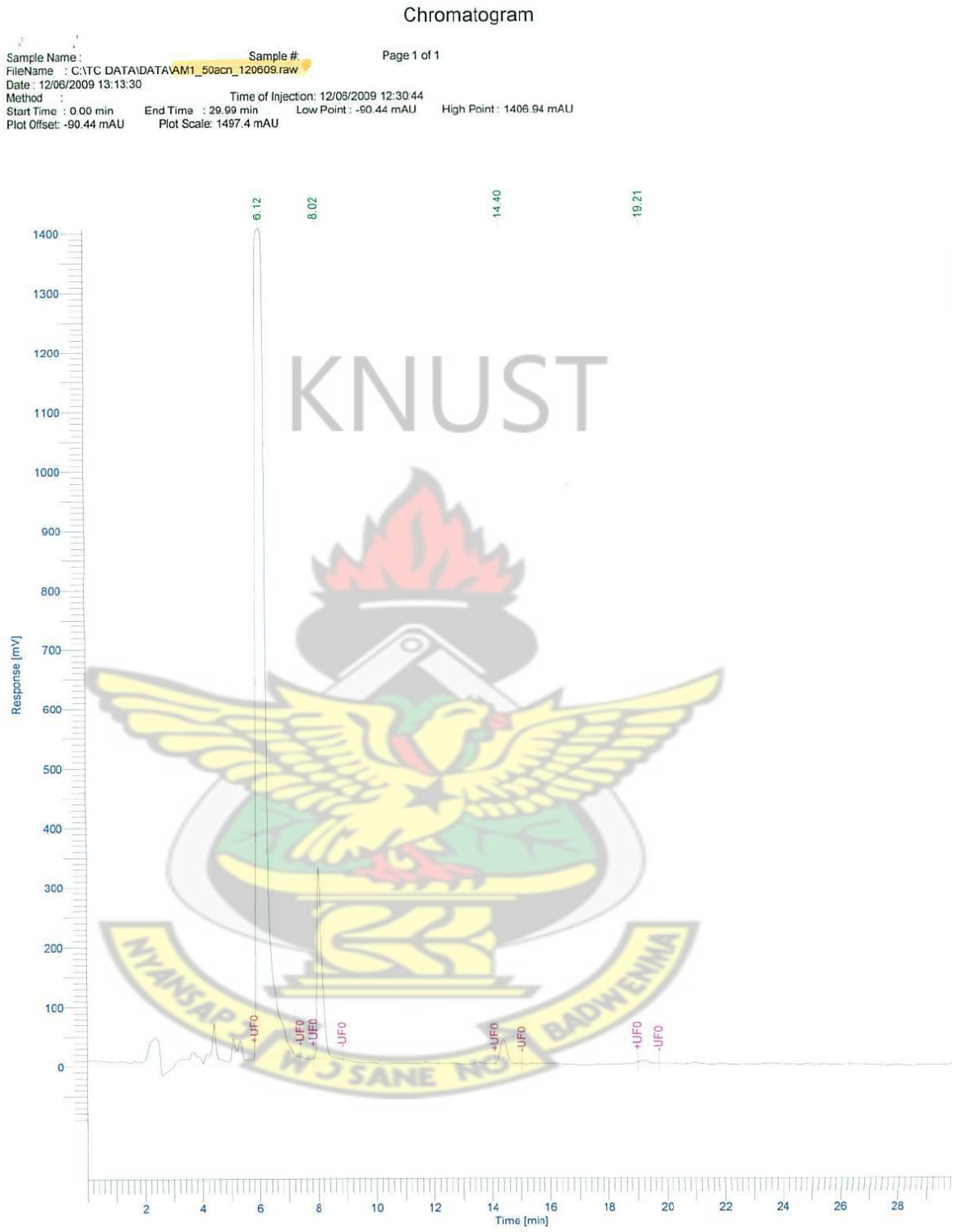
APPENDICES

APPENDIX 1: Plants with termite control properties (HDRA 2001)

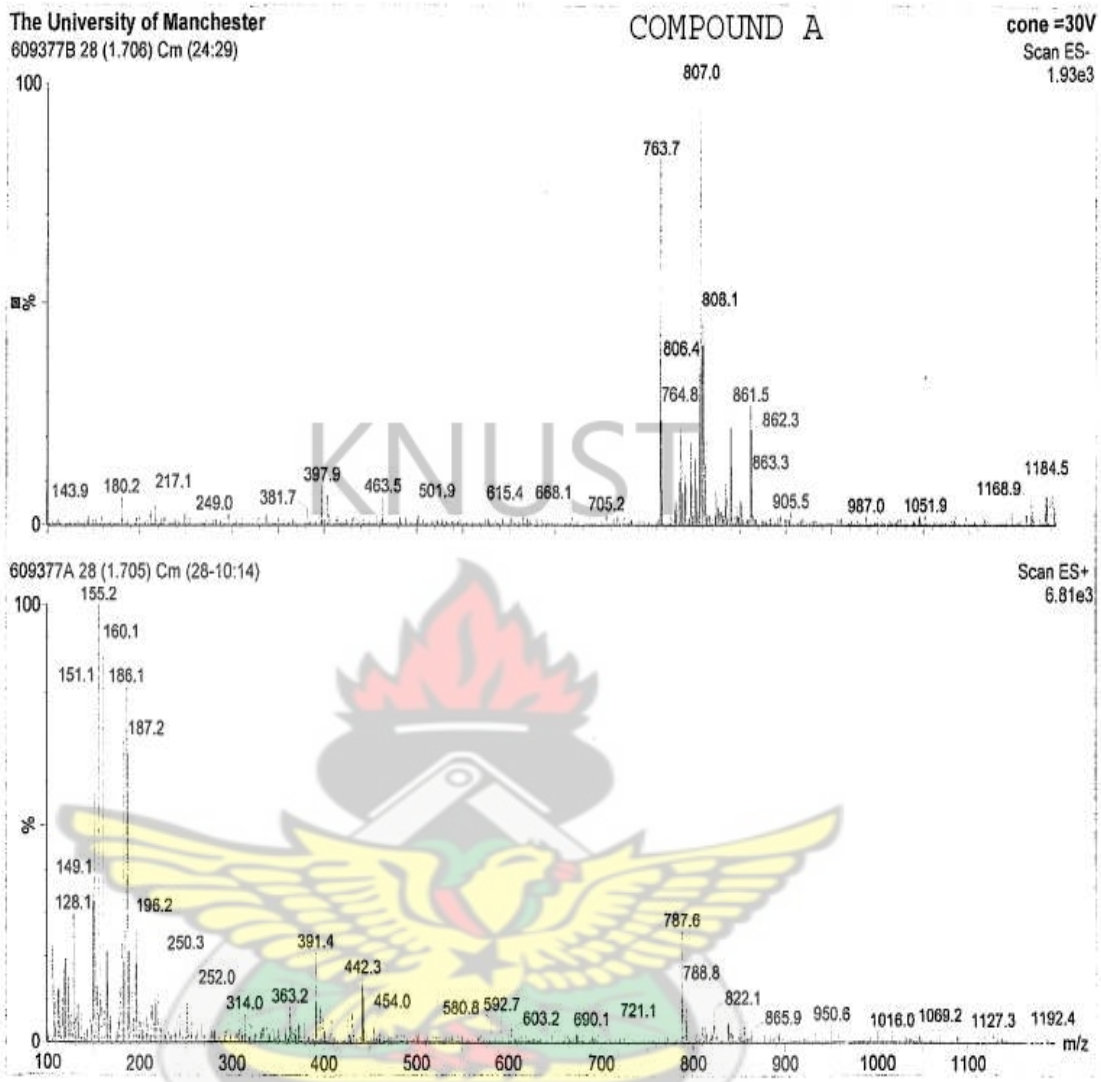
Species	Common name	Termite control property	Parts used
Acacia nilotica	Egyptian thorn	Anti-insect	Wood/pulp
Agave americana	American aloe	Repellent, insecticidal	Whole plant
Allium sativum	Garlic	Anti-feedant, bacterial, fungicidal, repellent	Bulbs
Anacardium occidentale	Cashew	Anti-insect, repellent	Seeds, oil
Argemone mexicana	Mexican poppy	Insecticidal, repellent	Whole plant
Azadirachta indica	Neem, nim	Termiticidal, anti-feedant	Leaves, seeds
Bidens pilosa	Blackjack	Anti-feedant, insecticidal, repellent	Whole plant, mature seeds
Calatropis procera		Termiticidal	Latex
Carya ovata	Shagbark hickory	Termiticidal	Bark
Camellia sinensis	Tea	Anti-feedant, insectidical	Leaves and fruit
Carica papaya	Pawpaw	Insecticidal	Fruit, fresh leaves and roots
Cassia siamea	Yellow cassia, kassof tree	Repellent	Used as a leaf mulch
Cedrela odorata	West Indian cedar	Termiticidal	Wood
Chemopodium ambrosioides	Wormseed	Anti-feedant, insecticidal, repellant	Whole plant
Consolida regalis	Blue cloud	Termiticidal	Seeds
Diospyros ebenum	Ebony	Anti-insect	Roots
Hyptis spicigera	Labiatae	Repellent	Aerial parts
Juniperus virginiana	Eastern red cedar	Anti-insect	
Leucaena leucocephala	Ipil ipil	Repellent	Used as a leaf mulch
Melia azedarach	Chinaberry, persian lilac	Anti-feedant, contact poison, repellant	Bark, branches, leaves, fruit, oil
Ocimum basilicum	Sweet basil	Insecticidal, repellent	Whole plant
Ocimum canum	Wild basil	Insecticidal, repellent	Whole plant
Ocimum urticifolium	Basil		Water-based extracts
Quercus prinus	Chestnut oak	Termiticidal	Bark
Samadera indica		Termiticidal	Leaves
Santalum album	Sandalwood	Anti-insect	
Tagetes minuta	Mexican marigold		Water-based extracts
Tectona grandis	Teak	Repellent	Wood/pulp

APPENDIX 2: Trees and shrubs with termite resistance (HDRA 2001)

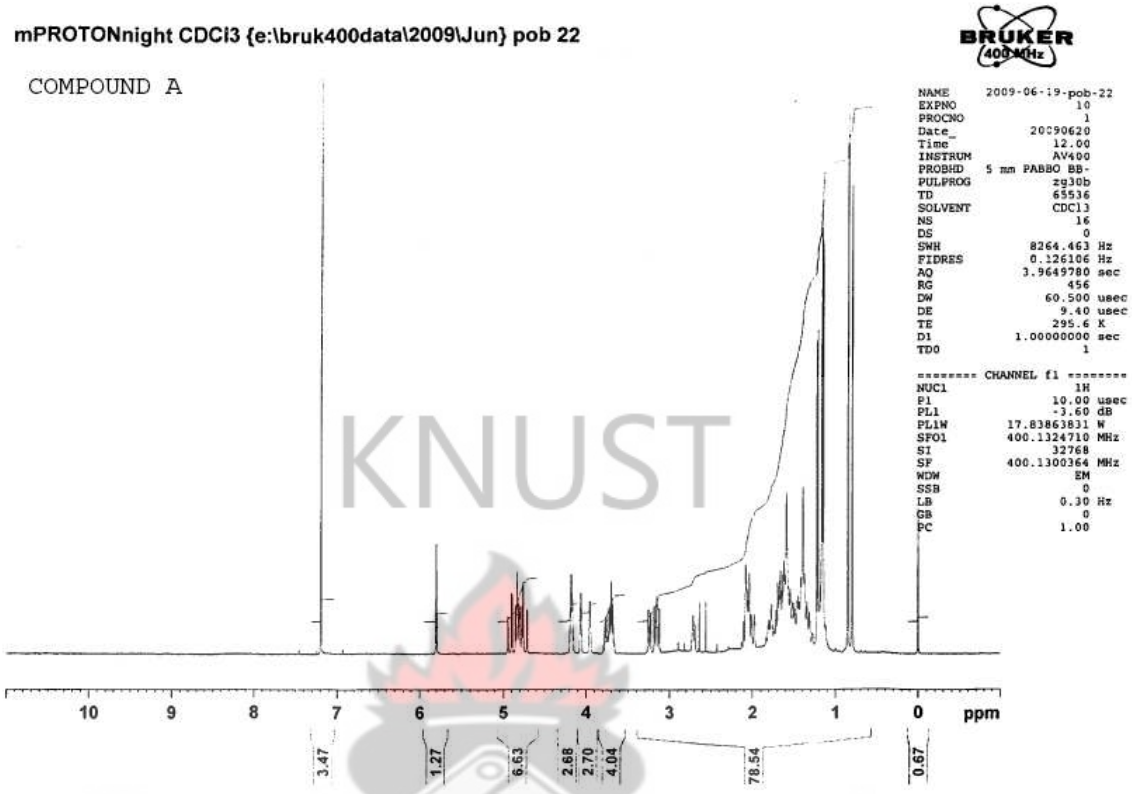
Species	Common name	Termite resistant part
Acacia polyacantha	Hook thorn	
Afrormosia laxiflora		Wood/pulp
Albizia odoratissima	Tes shade tree	Wood/pulp
Albizia zygia		
Azadirachta indica	Neem, nim	
Borassus aethiopum	African fan palm	
Brachylaena hutchinsii	Muhugu oil tree	
Capparis aphylla		Wood/pulp
Catalpa bignonioides	Common catawpa	
Cedrus deodora	Himalayan cedar	Wood/pulp
Daniellia oliveri		Gum/resin
Detarium senegalense		Wood/pulp
Dodonaea viscosa	Purple hop bush	Wood/pulp
Erythropleum suaveolens		Wood/pulp
Eucalyptus microcorys		
Grevillea robusta	Silky oak, silver oak	
Juniperus procera	E. African pencil cedar	
Melia azedarach	White cedar	Wood/pulp, leaves, seeds, oil
Strychnos nux-vomica		Leaves
Zanthoxylum xanthoxyloides		Wood/pulp



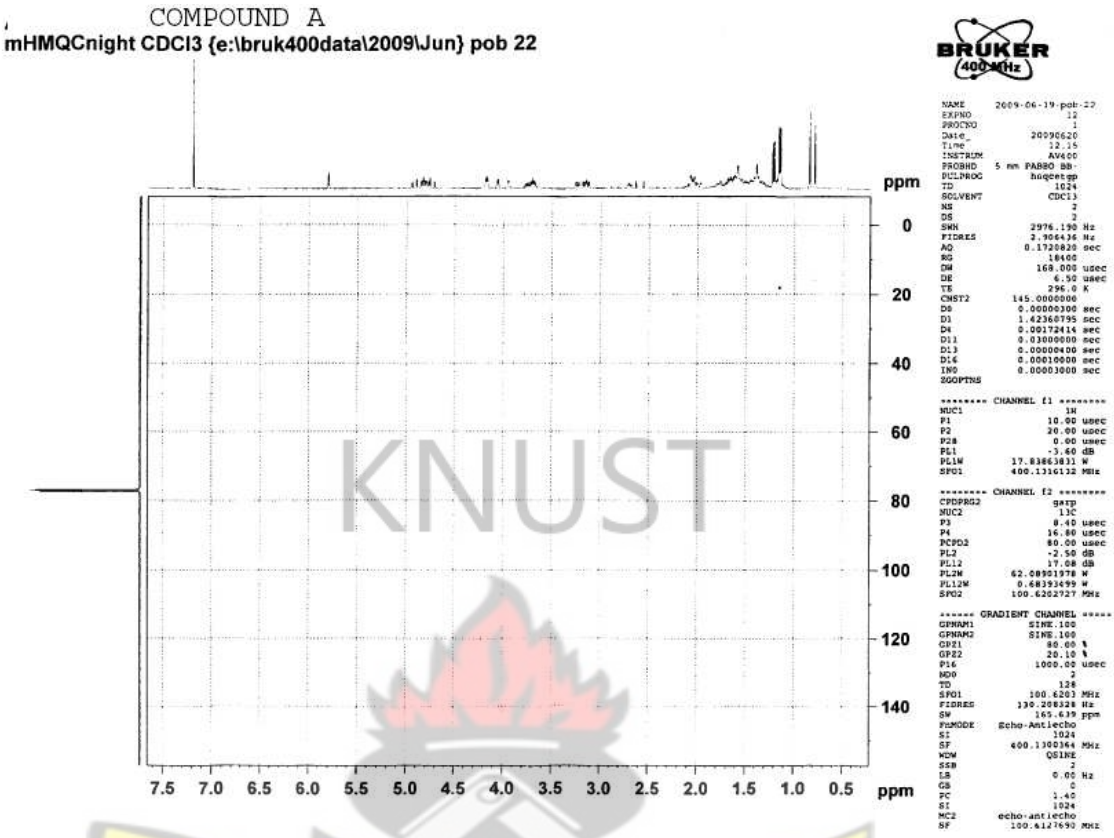
APPENDIX 3: HPLC CHROMATOGRAM OF FRACTION AM1



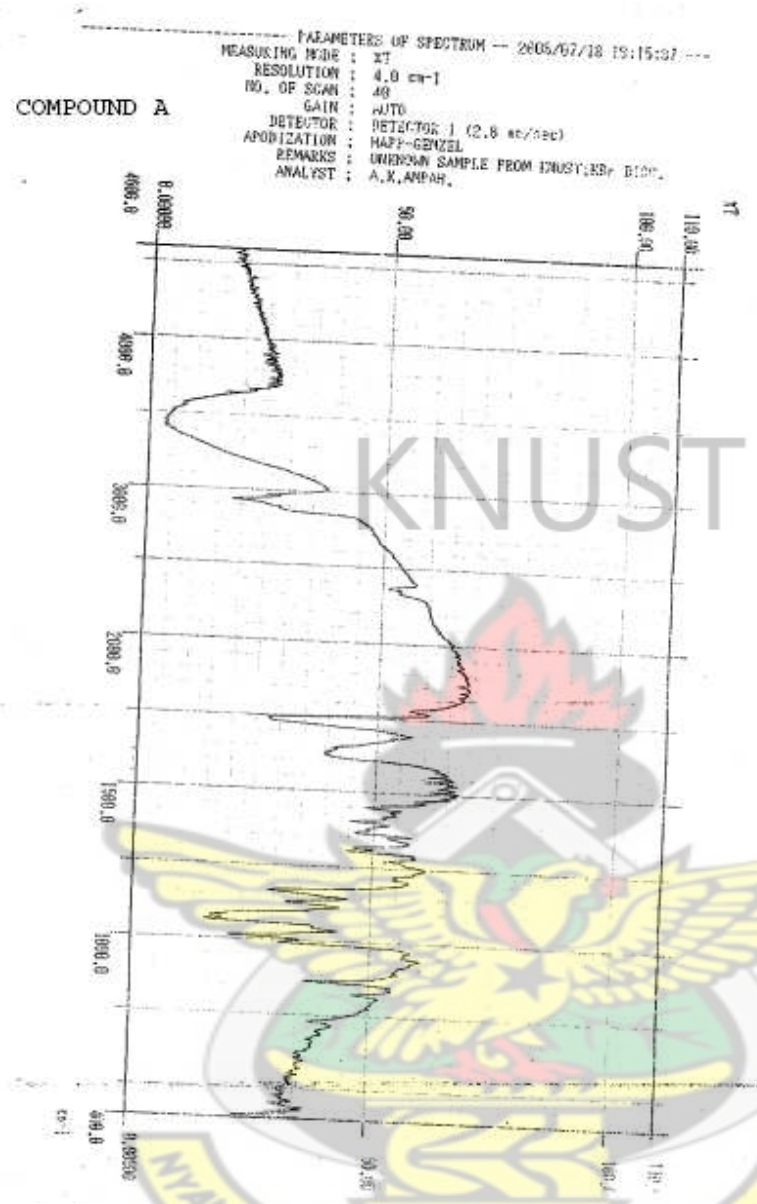
APPENDIX 4: MASS SPECTRA OF COMPOUND A



APPENDIX 5: ^{13}C NMR SPECTRUM OF COMPOUND A

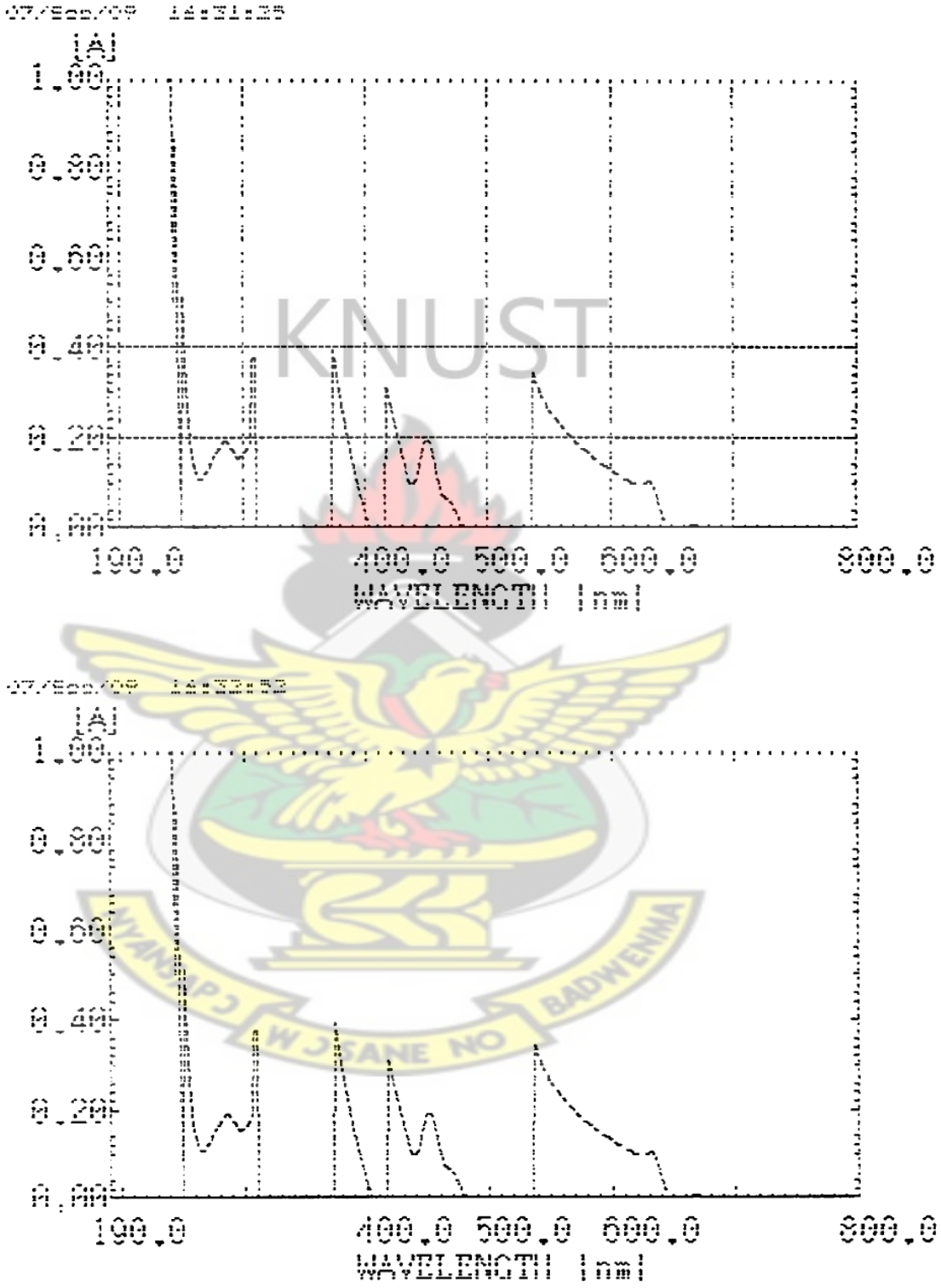


APPENDIX 6: ^{1}H NMR SPECTRUM OF COMPOUND A

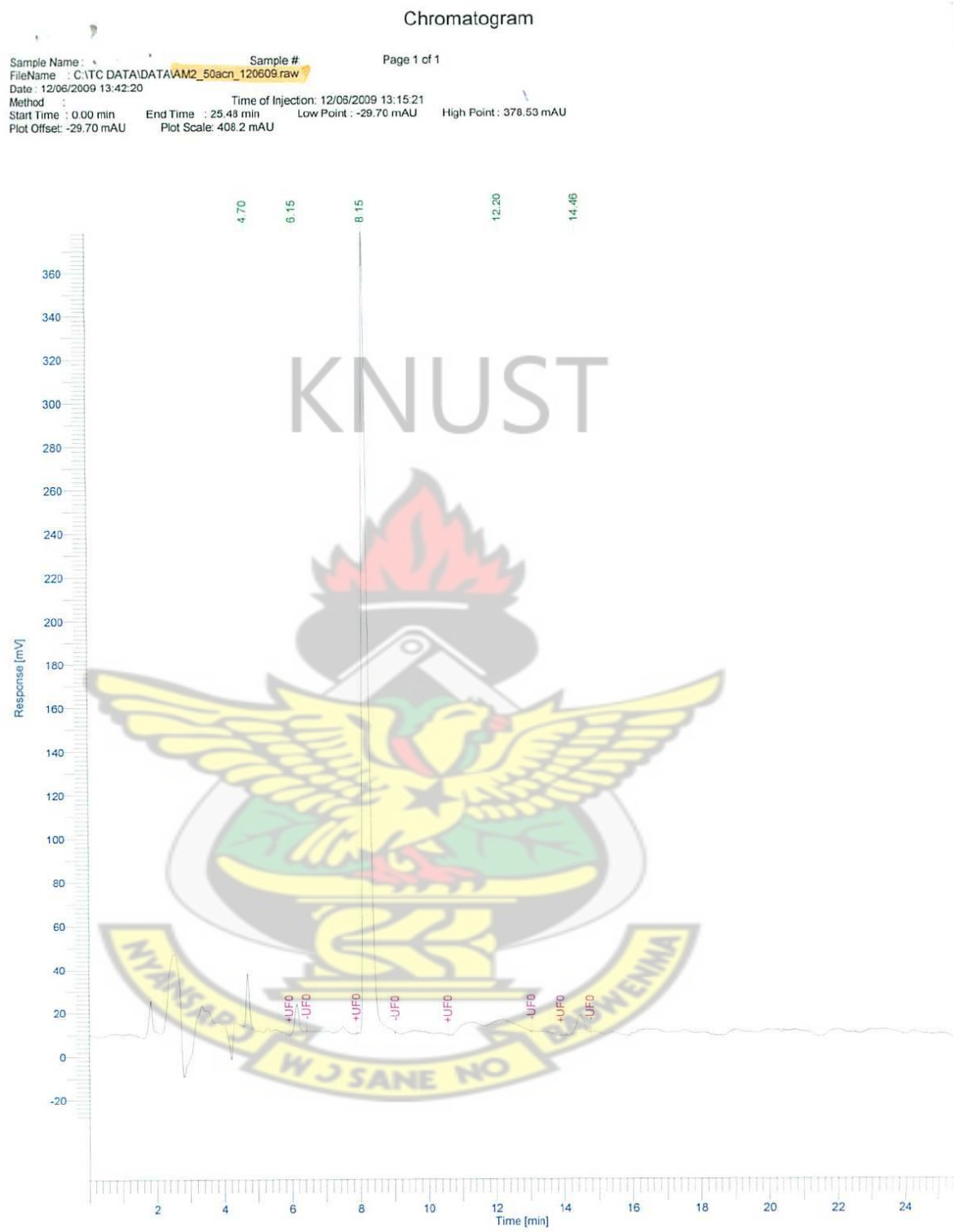


APPENDIX 7: INFRA RED SPECTRUM OF COMPOUND A

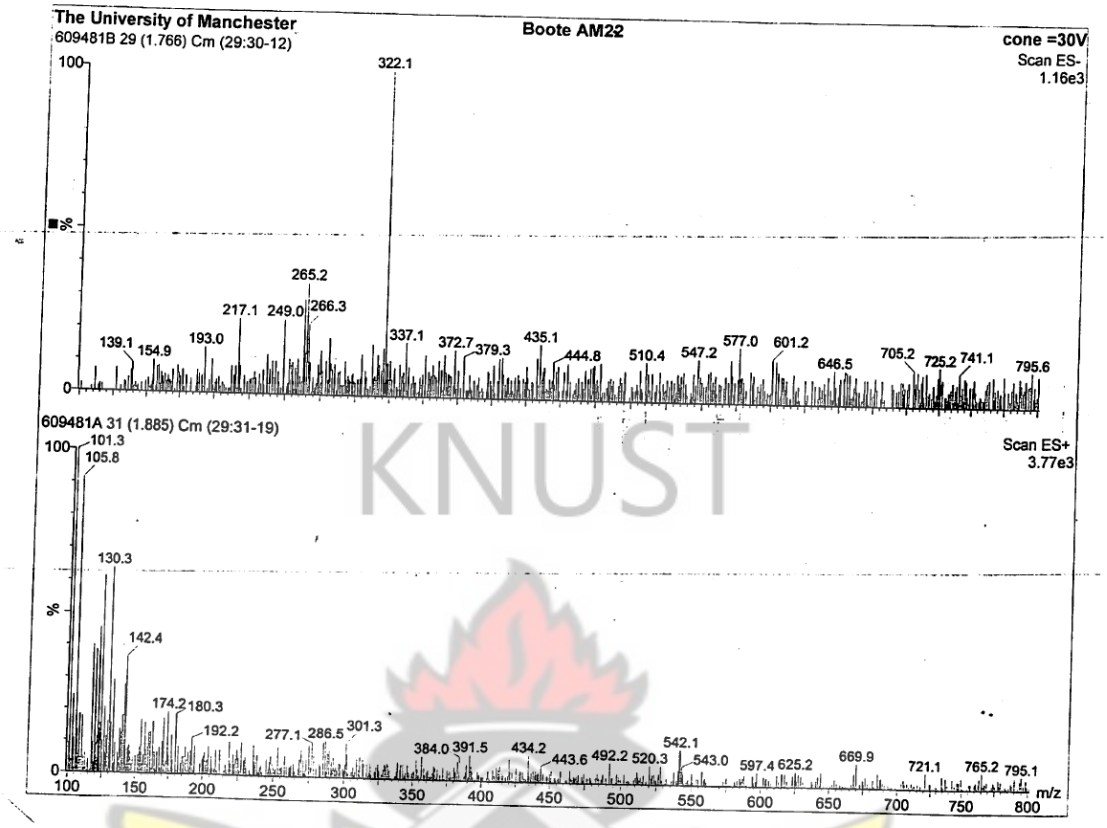
COMPOUND A



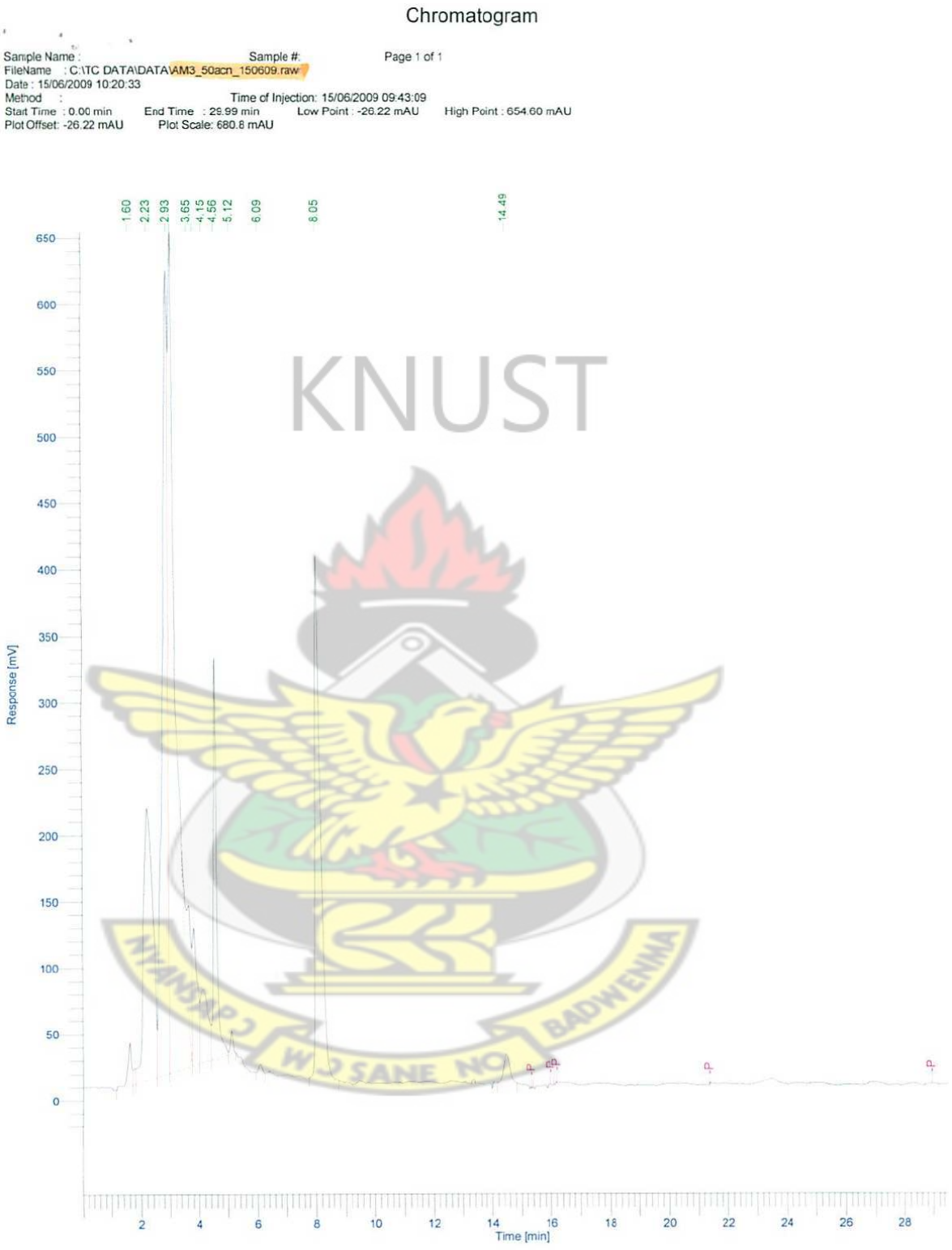
APPENDIX 8: ULTRA VIOLET SPECTRUM OF COMPOUND A



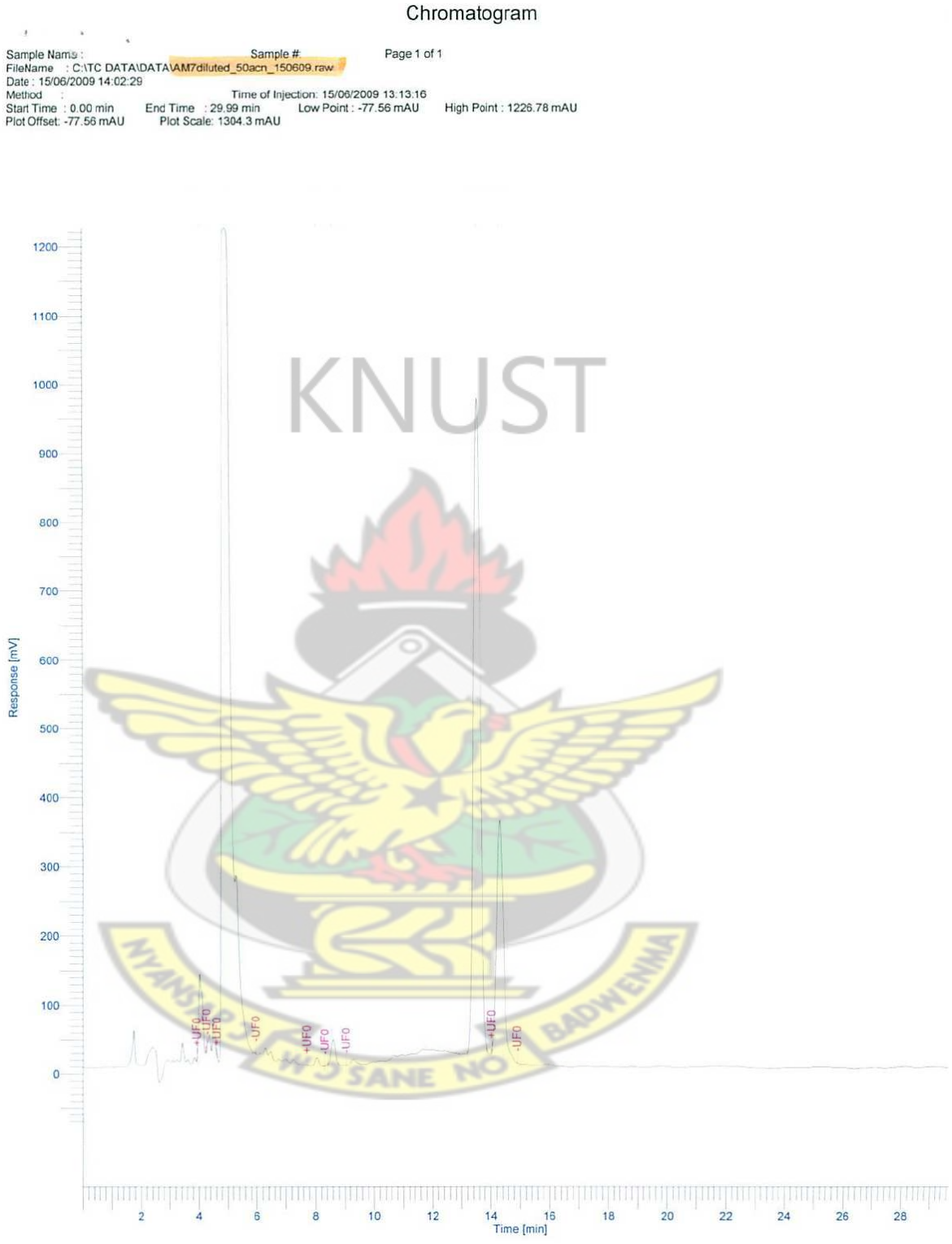
APPENDIX 9 : HPLC CHROMATOGRAM OF AM2



APPENDIX 10: MASS SPECTRA OF COMPOUND 22



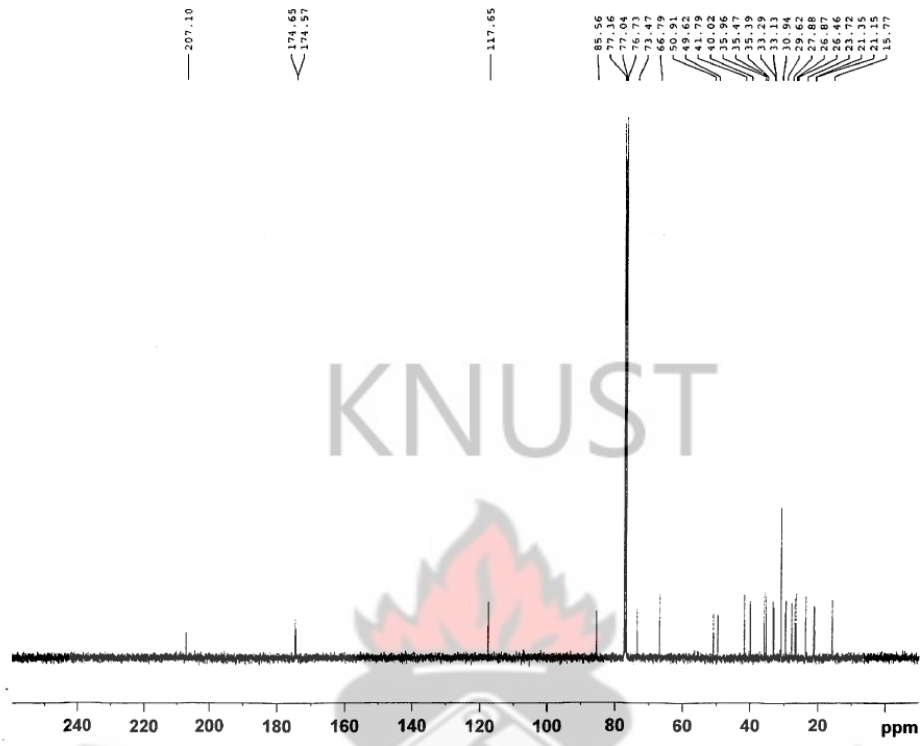
APPENDIX 11: HPLC CHROMATOGRAM OF AM3



APPENDIX 12: HPLC CHROMATOGRAM OF FRACTION AM7

COMPOUND B

mCARBONnight CDCl3 {e:\bruk400data\2009\Jun} pob 20

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PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                         256
DS                           2
SWH                  30241.936 Hz
FIDRES               0.461455 Hz
AQ                   1.0835786 sec
RG                         512
DW                      16.533 usec
DE                       7.83 usec
TE                      295.6 K
D1                   2.00000000 sec
D11                  0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        13C
P1                        8.00 usec
PL1                       0.00 dB
PL1W                34.91522217 W
SFO1               100.6241042 MHz

======== CHANNEL f2 ========
CPDPRG2                 waltz16
NUC2                        1H
PCPD2                    90.00 usec
PL2                      -3.60 dB
PL12                     15.31 dB
PL13                     18.00 dB
PL2W                17.83863831 W
PL12W                0.22927761 W
PL13W                0.12341322 W
SFO2               400.1316005 MHz
SI                        32768
SF                 100.6127690 MHz
WDW                          EM
SSB                           0
LB                        1.00 Hz
GB                            0
PC                        1.40
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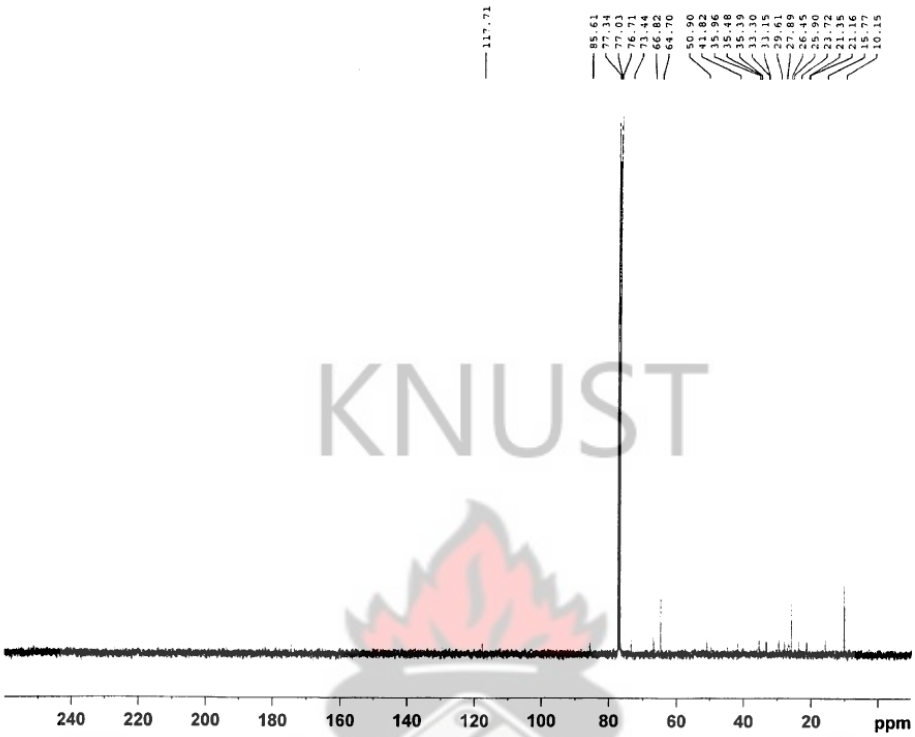
APPENDIX 13 ^{13}C NMR OF COMPOUND B

COMPOUND B

mCARBONnight CDCl3 {e:\bruk400data\2009\Jun} pob 21



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TD	65536
SOLVENT	CDCl3
NS	256
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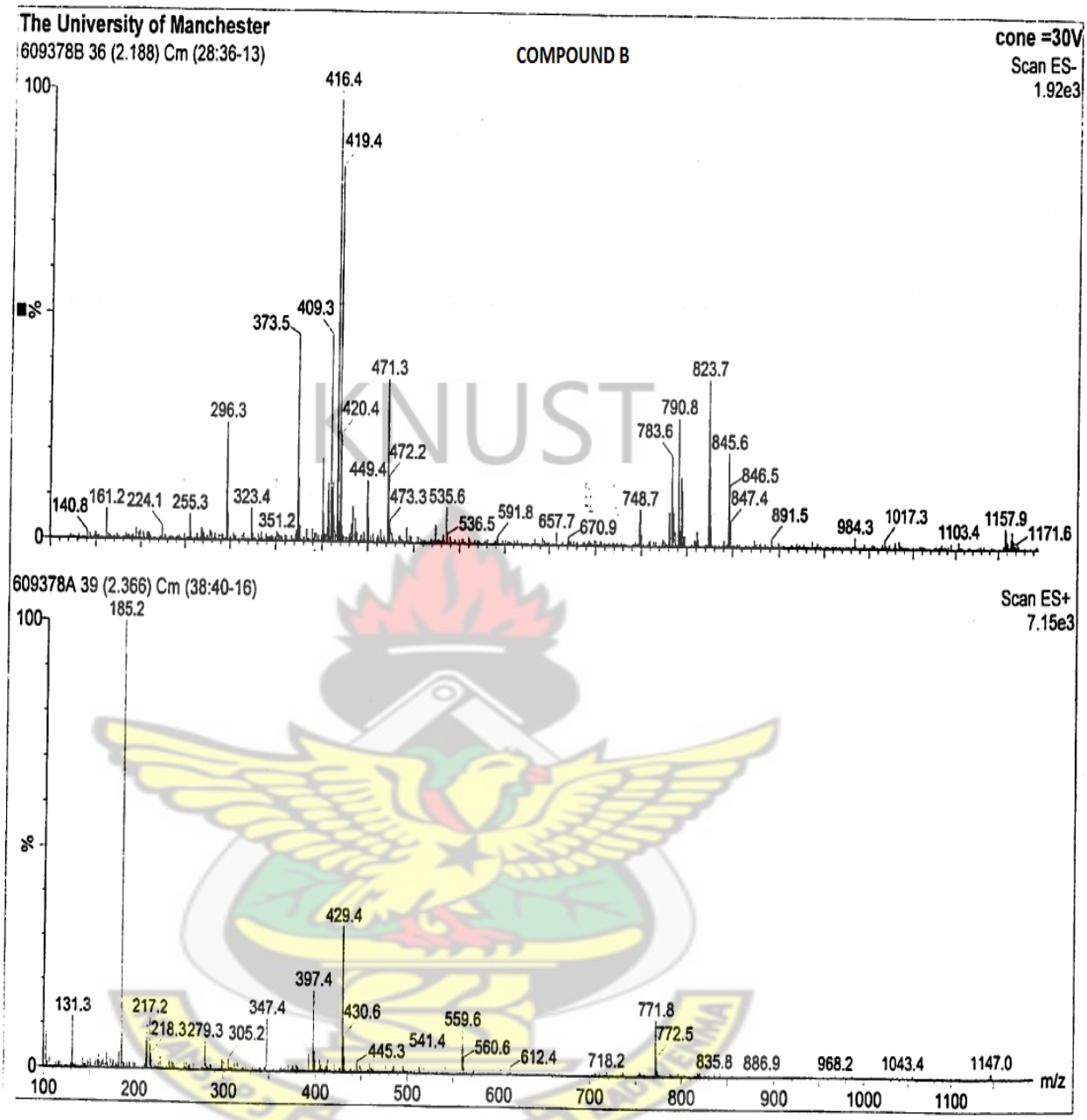
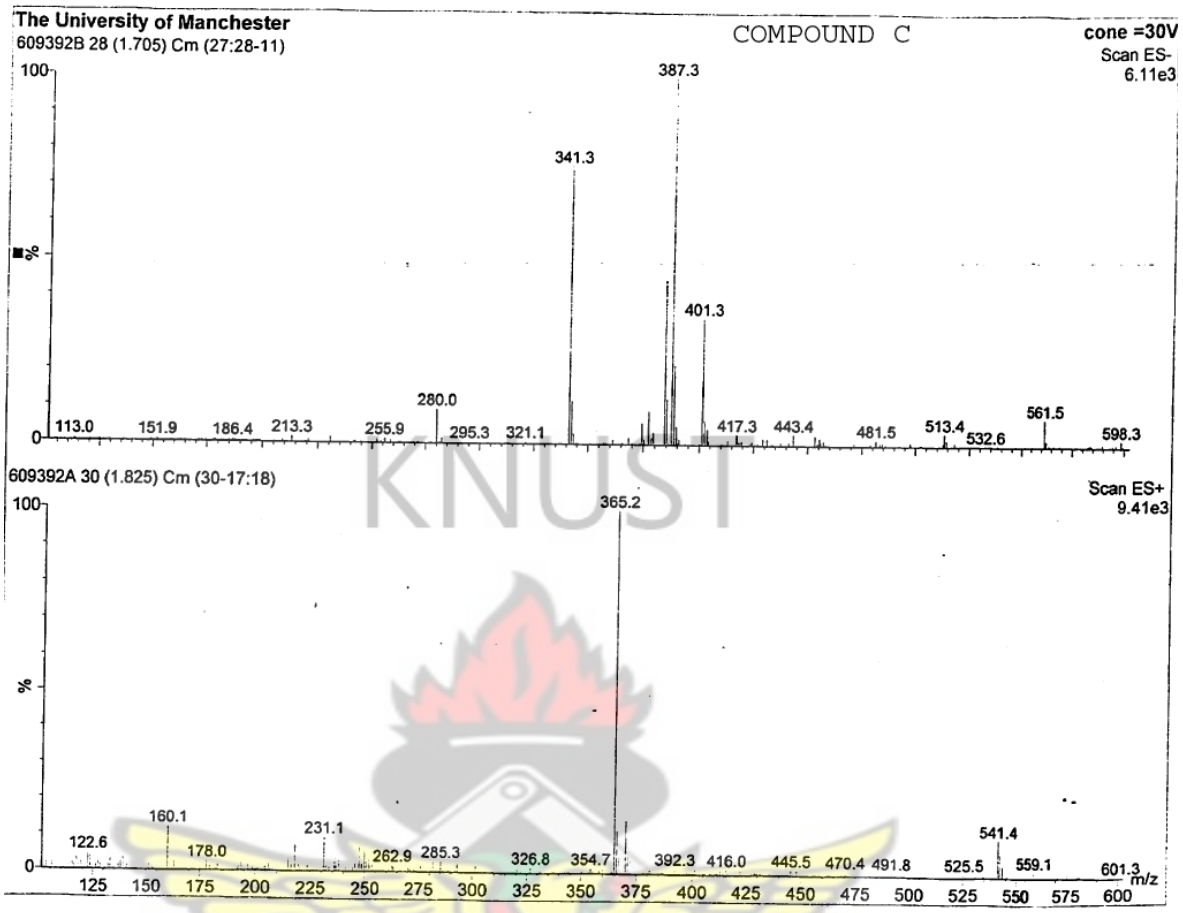
SWH	30241.936 Hz
FIDRES	0.461455 Hz
AQ	1.0835786 sec
RG	512
DW	16.533 usec
DE	7.83 usec
TE	295.6 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	8.00 usec
PL1	0.00 dB
PL1W	34.91522217 W
SFO1	100.6241042 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-3.60 dB
PL12	15.31 dB
PL13	18.00 dB
PL2W	17.81863831 W
PL12W	0.22927761 W
PL13W	0.12341322 W
SFO2	400.1316005 MHz
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

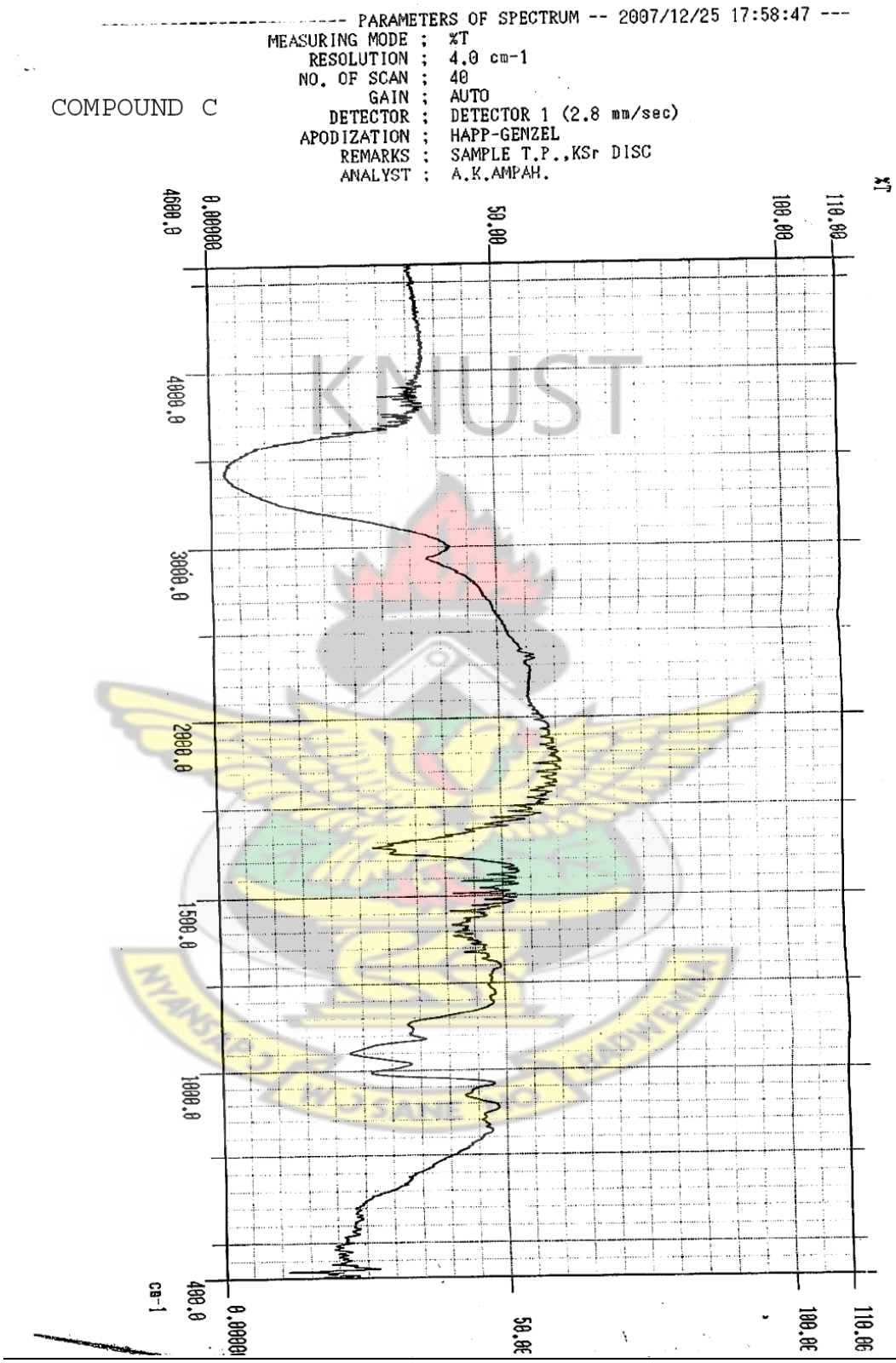
APPENDIX 14: ^{1}H NMR OF COMPOUND B



APPENDIX :MASS SPECTRA OF COMPOUND B



APPENDIX 16: MASS SPECTRUM OF COMPOUND C



APPENDIX 17: UV SPECTRUM OF COMPOUND C

APPENDIX 18: ANALYSIS OF VARIANCE FOR EXPERIMENT 1

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treat 2	6	92.4902	15.415	15.639	<.0001
Error	140	137.9948	0.9857		
C. Total	146	230.485			
Means for Oneway Anova					
Level	Number	Mean	Std Error	sig	% dam
HS	21	4.01951	0.21665	a	57.1429
VZL	21	3.86965	0.21665	a	56.1905
CN	21	3.83782	0.21665	a	54.2857
CG	21	3.70853	0.21665	a	53.2381
VZR	21	3.63762	0.21665	a	44.7619
CON	21	3.60658	0.21665	a	42.8571
TP	21	1.54535	0.21665	b	12.381

APPENDIX 19: ANALYSIS OF VARIANCE FOR EXPERIMENT 2

Analysis of Variance						
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	
treat	4	31.62407	7.90602	3.1648	0.019	
Error	70	174.8669	2.4981			
C. Total	74	206.4909				
Means for One way Anova						
Level	Number	Mean	Std Error	sig		Mean % dam)
COS	15	3.47378	0.40809	a		42.67
CP	15	3.31542	0.40809	a		42.67
COL	15	2.99597	0.40809	ab		36.00
CONTROL	15	1.9792	0.40809	b		24.00
TP	15	1.96889	0.40809	b		17.33

APPENDIX 20: ANALYSIS OF VARIANCE FOR EXPERIMENT 3

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treat	3	19.00087	6.33362	2.9033	0.0427
Error	56	122.166	2.18154		
C. Total	59	141.1669			
Means for Oneway Anova					
Level	Number	Mean	Std Error	sig	% dam
JR	15	1.96131	0.38136	a	17.3333
JS	15	1.01484	0.38136	a	9.3333
CON	15	0.90187	0.38136	a	6.6667
TP	15	0.40594	0.38136	b	2.6667

APPENDIX 21: ANALYSIS OF VARIANCE FOR EXPERIMENT 5

Oneway Anova					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
treat	4	20.41391	5.10348	16.7954	<.0001
Error	70	21.27036	0.30386		
C. Total	74	41.68427			
Means for Oneway Anova					
Level	Number	Mean	Std Error		Mean
R1	15	0.52889	0.14233	a	8
R2	15	0	0.14233	a	0
S1	15	0.52889	0.14233	a	8
S2	15	1.25149	0.14233	b	29.3333
c	15	1.42026	0.14233	b	33.3333
Std Error uses a pooled estimate of error variance					

APPENDIX 22: ANALYSIS OF VARIANCE FOR EXPERIMENT 6

Analysis of Variance						
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	
treat	6	360.4236	60.0706	2855.9	<.0001	
Error	98	2.06135	0.021			
C. Total	104	362.4849				
Means for One way Anova						
Level	Number	Mean	Std Error	sig	Mean (% dam)	
WA1	15	4.319	0.037	a	74.67	
PET2	15	4.111	0.037	a	60.00	
PET 1	15	4.062	0.037	a	58.67	
ET1	15	4.005	0.037	a	54.67	
WA2	15	3.978	0.037	a	53.33	
ET2	15	0.000	0.037	b	0.00	
DU	15	0.000	0.037	b	0.00	

APPENDIX 23:ANALYSIS OF DATA COLLECTED ON LABORATOTY TESTING OF EXTRACT OF *T.PERUVIANA*

	Termite mortality					
Extract/hours after exposure	2	4	6	8	20	Total
CONTROL	2	2	2	2	7	15
PET ETHER EXT	3	5	6	6	31	51
ETHANOL EXT	5	7	11	20	38	81
WATER EXT	0	1	3	3	21	28
Total	10	15	22	31	97	175

Cells	Contribution to symmetry chi-squared
n1_2 & n2_1	0.2
n1_3 & n3_1	1.2857
n1_4 & n4_1	2
n1_5 & n5_1	7
n2_3 & n3_2	0.0769
n2_4 & n4_2	3.5714
n2_5 & n5_2	31
n3_4 & n4_3	12.5652
n3_5 & n5_3	38
n4_5 & n5_4	21

	chi2	df	Prob>chi2
Symmetry (asymptotic)	116.7	10	0.000
Marginal homogeneity (Stuart-Maxwell)	109.86	4	0.000