EFFICACY OF EXTRACTS OF *Datura metel* L. AND *Euphorbia hirta* L. AS PRESERVATIVES FOR BAMBOO (*Bambusa vulgaris*) AGAINST INSECT AND FUNGI ATTACKS

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DEDICATION

The work is dedicated to God Almighty, the one who was, who is and always will be.

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ABSTRACT

Lignocellulosic materials are susceptible to bio-degradation. Therefore, preservatives are used to enhance their durability for various applications. The toxic nature of synthetic preservatives is encouraging the use of bio-preservative. Bio-preservatives from lignocellulosic extracts though a viable alternative are neither readily available nor their efficacy fully known. This study was therefore designed to evaluate the efficacy of extracts of *Datura metel (Dm)* and *Euphorbia hirta (Eh)* as bio-preservatives for *Bambusa vulgaris (Bv)* against termite and fungi attacks as well as its effect on the laminated board strength.

The *Dm*, *Eh* and *Bv* samples were sourced in Ejiooku, Akinyele Local Government, Ibadan, Nigeria, and authenticated in Herbarium of the Forestry Research Institute of Nigeria. Phytochemical screening and characterisation of the extracts were determined by gas chromatography and mass spectrometry. Chemical constituents of the extracts were determined using AOAC procedures. Bio-preservatives were formulated from extracts at 0, 25, 50, 75 and 100% concentrations using ASTM procedures. The *Bv* culms were sampled from top, middle 1, middle 2 and base, cut into strips (60×20×4 mm) and soaked in the preservatives for 72 hours. Treated samples and control were sub-divided into A, B and C groups. Group A was subjected to termite *(Macrotermis bellicosus)* attacks for 12 months, while groups B and C were subjected to brown *(Sclerotium rolfsii)* and white rot *(Pleurotus florida)* fungi for 6 months in accordance with ASTM procedures. Weight Loss (WL) analyses were done to evaluate the potency of the formulated bio-preservatives, while its effect on gluability of Bamboo Laminated Block (BLB) were also investigated using ASTM procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Flavonoids (0.11 and 0.12 mg/g), saponins (0.31 and 0.28 mg/g) and tannins (0.04 and 0.03 mg/g) were obtained from *Dm* and *Eh*, respectively. Bicyclo [3.1.1] heptanes, 2, 6, 6-trimethyl (1α, 2β, 5α) were major chemical constituents in the two extracts. The presence of these metabolites and chemical constituents formed the basis for their utilisation as preservatives. The WL in group A ranged from 0.62±0.29 to 0.83±0.50 and 0.77±0.40 to 0.96±0.61 for *Dm*- and *Eh*-treated samples, respectively, indicating that treated samples resisted termite attack better than control (1.75±0.57). The group B WL ranged from 1.22±0.42 to 1.89±0.33 and 0.76±0.58 to 1.86±0.69 for *Dm* and *Eh*-treated samples, respectively. Those of group C ranged from 0.16±0.08 to 0.81±0.51 and 0.28±0.24 to 0.71±0.33 for *Dm* and *Eh*-treated samples, respectively. This implied that treated samples were adequately protected against brown and white rot fungi attack compared to control. There were significant variations in WL from top (1.89±0.33) to base (1.25±0.40), showing that fungi attacks were least at the base and highest at the top. Shear strength for BLB ranged from 6.06 to 3.85 N/mm and 5.13 to 4.44 N/mm for control and treated samples, respectively, indicating the suitability of the preservatives as there were no significant differences.

The bio-preservatives formulated from *Datura metel* and *Euphorbia hirta* extracts offered adequate protection to bamboo against termite and fungi attacks. They are suitable preservatives for lignocellulosic materials.

Keywords: Bio-preservatives, *Bambusa vulgaris*, Lignocellulosic materials, *Datura metel* and *Euphorbia hirta* extracts.

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TABLE OF CONTENTS

List of Table

List of Plate

List of Figure

Appendix

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Wood, an adaptable material, have been utilised for housing erection, furniture, tools making, energy, etc. The rate of timber utilisation, technological breakthrough, lignocellulosic materials deterioration, forest over exploitation and citizenry explosion worldwide have led to an incredible increase in demand for wood (Fuwape, 2000). Sekumade and Oluwatayo (2011) remarked that the demand for wood is increasing in Nigeria with an attendant dwindling supply (Lucas, 1983; Onilude, 2006; Lucas, *et al* 2006; Adewole and Olorounnisola, 2010). Also, the scarcity of choice timber such as Obeche, Mahogany, Iroko, Afara, Sapele Mahogany, Mansonia, Agba, among others, with increasing price of timber species is necessitating the need for alternative raw materials in structural and applications.

Bamboo, a woody grass is a candidate raw material that has been used structurally as scaffoldings and in the construction of bridges (Correal, 2020). Bamboos are a diverse group of mostly evergreen perennial flowering plants making up the subfamily Bambusoideae of the grass family Poaceae. It has over 1,500 species worldwide, grouped into about 70 genera (Khalil *et al* 2012). It grows abundantly in almost all tropical countries as a blooming species which allows reaping for utilisation about 4-7 years (Ashby, 2016).

According to Akinlabi *et al* (2017), bamboo has been recognised as one of the oldest construction materials used for flooring, ceiling, walls, housing roofs, doors, windows, fences, rafters and trusses. However, bamboo has a lot of problems associated with its utilisation, the major one being that the woody stem (culm) has relatively high starch content, making it particularly vulnerable to termite and fungal assault.

Consequently, it becomes imperative that bamboo culms be given a treatment before being used to lengthen the life of the item.

Preservatives are pesticides and fungicides that protect lignocellulosic materials including bamboo against fungi, bacteria, or insects attack. Synthetic preservatives are broad-spectrum chemicals that cause threats to human beings primarily due to the increased disposal problems associated with their uses during or after preservation. As a result, there has been restriction or outright ban of some common non-biodegradable preservatives. This necessitates research into development of environmentally benign, preservatives as alternatives to the hazardous synthetic ones (Mohsen *et al* 2011).

Vanneste *et al* 2002 and Maoz *et al* 2007 observed that, research has geared recently towards the possibility of using extracts derived from medicinal plants (leaves, seeds, fruits wastes and essential oils) as insecticides and fungicides to preserve lignocellulosic materials all over the world. This is because they are relatively inexpensive, biodegradable and environmentally friendly. Bio-extracts are generally divided into the aliphatic and alicyclic categories, respectively, (i.e., flavonoids and tannins) (terpenes and terpenoids) and phenolic substances. The types of active molecules make up in these extracts determines their antifungal effectiveness, which depend on a variety of methods, such as direct contact with fungal enzymes, damage to cell membranes and cell walls that causes leakage of the cell's contents or a disruption of ion homeostasis, or antioxidant activity (Singh *et al* 2012; Valette *et al* 2017).

There is therefore the need to investigate the inherent pesticide and fungicide potentials of indigenous herbaceous plants to broaden the lists of existing environmental friendly biocides that have been confirmed suitable for wood preservation (Kriti *et al* 2021).

1.2 Statement of Problems

Freshly felled bamboo culms are generally prone to damage from termites and fungi, which may reduce the material into a powdery mass within a limited period of time. Borer infestation is a serious problem in bamboo growing countries (Bhat *et al* 2005) coupled with biological decay caused by fungi. The service life of an untreated bamboo

can range from a number of days to a little week, months or years determined by the conditions or location of use, species, age, etc. An enormous quantity of bamboo is lost every year resulting in loss of substantial amount in financial, labour and time resources, because of its perishable nature (Maoz *et al*., 2007). Razak *et al* 2004 reported that it might be impossible to assess accurately these losses since records concerning them are rarely kept in many part of the world.

Synthetic preservatives have been used to preserve bamboo and this has been found to contain active ingredients such as creosote, arsenic, zinc, copper and chromium, among others (Ibach, 1999; Homan and Jorissen, 2004). Most of these chemicals are harmful to human, animals and the environment, aside from the fact that they are usually very expensive (Onuorah, 2000). There is need therefore to seek thoroughly for environmentally friendly preservatives that are less harmful or not poisonous to humans and not expensive as well as being able to successfully protect and improve the service life of bamboo.

Although reports revealed that certain extraneous materials from durable plant species have been extracted and formulated into bio-preservatives for treatment of less durable or perishable lignocellulosic materials and found to be effective. Despite reports (Sharma, 2002 and Bajwa *et al* 2008) on the antimicrobial, anti-termite and antifungi properties of extracts from *Datura metel* and *Euphorbia hirta*, literature is sparse on the efficacy of their extracts in the control of termites and fungal activities on bamboo culms.

1.3 Aim and Objectives

The aim of the study was to formulate biodegradable and environmental benign preservatives from extracts of two selected plants with a view to finding alternatives to conventional synthetic preservatives.

The specific objectives of this investigation were to:

i. investigate the active metabolites and chemical constituents present in *Datura metel* and *Euphorbia hirta* extracts.

ii. assess the efficacy of the plant extracts for treatment of *B. vulgaris* against termite and fungi attacks

iii. investigate the impact of preservation treatment on the ability of laminated bamboo strips to adhere.

1.4 Justification

Over the years, efforts have been made to address and control activities of bio-deteriorating agents lignocellulosic materials. According to Anwar *et al* (2004) preservation has been effective in improving utilisation potentials of lignocellulosic materials in furniture and construction industry. Bamboo must be treated with preservatives for it to be adequately and efficiently used as a reliable construction raw substance and other industrial purposes.

Studies conducted by Kazemi *et al* 2006 and Adegeye *et al* 2009 have shown that perishable timber species can be preserved with extract derivative from non-perishable wood species. According to Sharma *et al* 2022 and Bajwa *et al* 2008 extracts of *Datura metel* and *Euphorbia hirta* possess antifungal properties that can destroy plant pathogens (*Colletotrichum capsici*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Phomopsis caricae-papayae*, and *Aspergillusniger*). It is therefore necessary to investigate the active metabolites and chemical compounds present in the extracts of *Datura metel* and *Euphorbia hirta* as potential insecticide and fungicide in order to supplement the bio-preservatives formulated from lignocellulosic extracts that are inadequate in supply.

Development of natural preservatives from extracts of the selected species will provide relevant information which will serve as a baseline for subsequent studies. Therefore, this research was done to evaluate the effectiveness of the formulated bio preservatives by using them for treatment of bamboo which is known to be prone to termite and fungi attack.

1.5 Scope of Study

The scope of the study covered the development of natural preservatives from *Datura metel* and *Euphorbia hirta* extracts for preserving *Bambusa vulgaris* against termite and fungi attacks. This involved determining the yield of each plant extracts, examining the metabolites and chemical constituents through phytochemical and gas chromatography screening of the plant extracts, developing bio-preservatives from the

extracts and evaluating the efficacy of the natural preservatives in preserving bamboo against termite and fungi attacks, as well as rate the performance of the bio-preservatives on gluing properties of bamboo laminated boards.

CHAPTER TWO
LITRATURE REVIEW

2.1 Bamboo

Ancient woody grass known as bamboo is found all throughout tropical, subtropical, and mild temperate regions. A significant non-wood forest product, due to the plant's very lengthy flowering cycles, bamboo taxonomy presents certain challenges for science. As a result, taxonomists continue to debate the overall number of bamboo species and genera. About 1200 species of bamboo in 90 genera are known to exist (Maxim, 2005). According to Olufemi (2003), more than one varieties of bamboo exist in Nigeria which include *Bambusa vulgaris* and *Oxystenanthera abyssynica*. The varieties attain average height ranging from 8 to 20 meter at full growth. In the south ward part of the River Niger and Taraba states, these species are invasive in their growth in the forests.

According to Borowski (2022), bamboo has an advantage over other woody materials due to its extraordinarily high biomass productivity; additionally, due to its rapid growth and greater rate of biomass accumulation, it may produce more fiber per hectare. Aside from its enormous biomass production, bamboo lacks rays or knots that would evenly distribute stress along its length (Li, 2004). The *Bambusa vulgaris* culm is very versatile both for industrial and household products, like food containers, skewers, chopsticks, handicrafts, toys, furniture, flooring, pulp and paper, boats, charcoal, musical instruments, weapons, bridges, scaffolding, and houses, also it is utilised in the manufacture of these items as well as others (Correal 2020)

Ogunsanwo and Terziev (2010) reported that laminate bamboo boards have stronger particular qualities than several premium timber types as *Khaya senegalensis, Mansonia altissima,* and *Milicia excelsa*. Although bamboo is a common material in Asian countries, it is not as common in African nations. For instance, despite the fact that bamboo is widely available, bonded items are not produced in Nigeria (Ogunsanwo *et al* 2015).

Plate 2.1: *Bambusa vulgaris* in Natural Forest
(Sources: https//en.m.org) 2017

2.1.1 Nigeria Bamboo Resources

Nigeria has 5 indigenous species *Bambusa arundinacea, Bambusa tulda, Dendrocalamus giganteus, Oxynanthera abyssinica* and *Bambusa vulgaris* (RMRDC, 2006, Ogunsile and Uwajeh, 2009) and the species *Bambusa vulgaris* is one of the most prevalent. It is an invasive plant that matures in 2 to 6 years and enormous towards the

south of Nigeria (Azeez *et al* 2016). Despite size variations, the species in Nigeria share comparable physical traits, indicating influences from age and maybe soil quality. Bamboo grows abundantly along river banks and in other generally swampy regions across Nigeria's rain forest belt, where it is especially well adapted (RMRDC, 1996), however it is sparse in Lagos and Bayelsa. Bamboo is present in profusion in all of the Southern Nigerian States of Abia, Ebonyi, Enugu, Anambra, Ogun, Oyo, Osun, Ondo, Edo, Delta, Rivers, Akwa Ibom, Cross River, and Imo States. In these states, bamboo makes up at least 10% of the natural vegetation, and existing bamboo clumps exhibit noticeable gregarious development that is contiguous over sizable expanses. In the states of Bayelsa, Kogi, Kwara, Benue,Lagos, Ekiti, as well as Nassarawa, bamboo makes up between 6.0 and 9.0% of the native vegetation. Along with Abuja, capital of Nigeria, there are also a few *bambusa vulgaris* cluster in the Plateau, Taraba, and Niger States. There are 12 states in which bamboo is not common (Plate 2.2). Katsina, Kebbi, Sokoto, Jigawa, Yobe, Adamawa, Bauchi, Borno, Gombe, Kano, Kaduna, as well as Zamfara are among them (RMRDC, 2004).

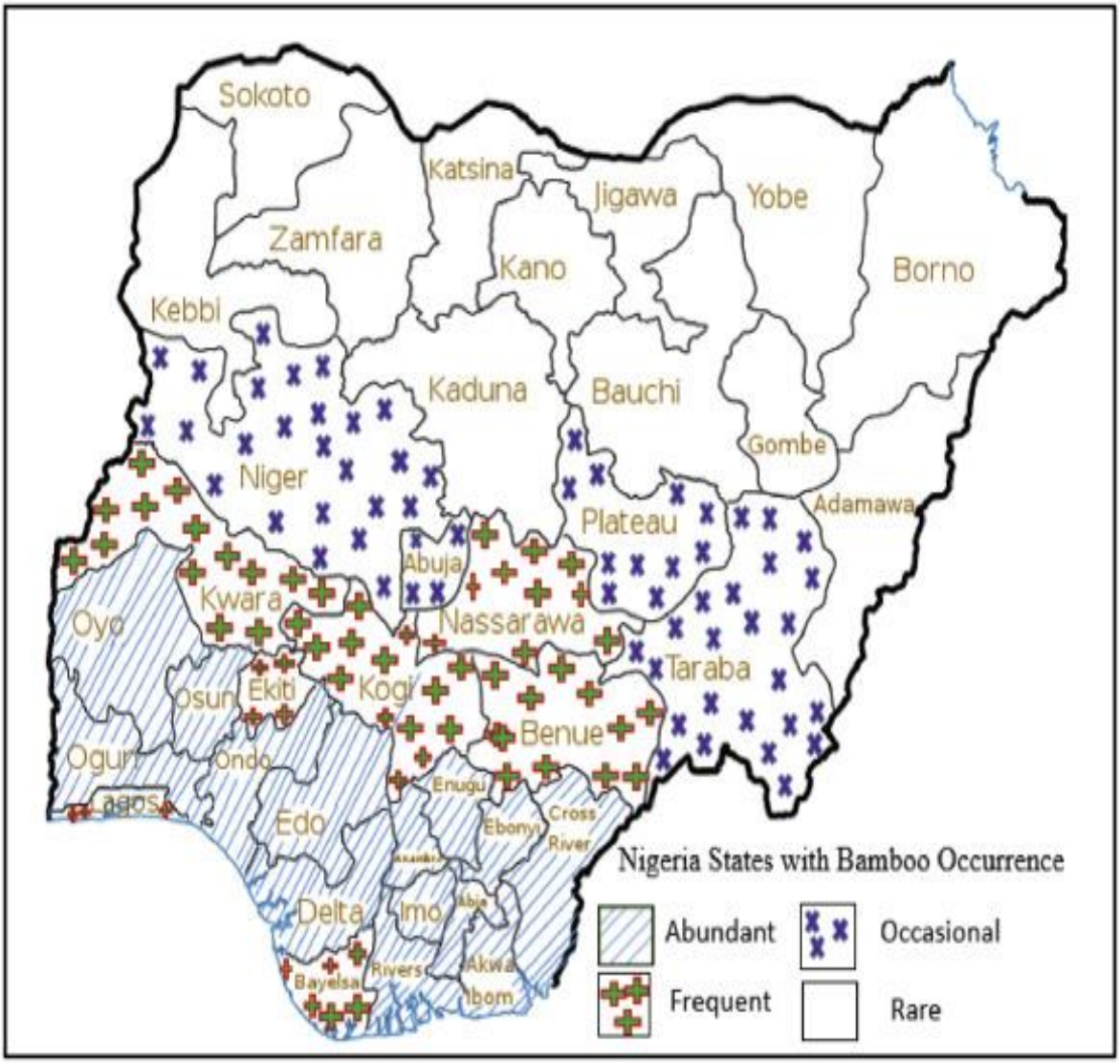

Plate 2.2: Vegetation Map of Nigeria Showing the Bamboo growth sites
(Source: http://www.researchgate.net) 2023

2.1.2 Physical and mechanical properties of Bamboo

The characteristics of bamboo are what determine its potential for industrial use. The austral structure of the anatomy determine a number of things, like the amount and arrangement of fibers about the vascular bundles and bamboo has a density that ranges around 500 to 800 kg/m^3 (Sattar, 1995). Usually, the density of a culm rises from its

center outward (Sekhar and Bhartari, 1960; Sharma and Mehra, 1970), also it increases from the bamboo culm's foot to its summit. At around 3 years old, the maximum culm density is reached (Liese 2015; Sattar and Kabir, 1990; Espisloy, 1994). Bamboo's physico-mechanical characteristics vary incredibly. They are more varied than those of timber in certain ways. The variance is brought on by uneven vascular bundle distribution, fluctuating moisture content, and variations in the physico-mechanical characteristics of the nodes and internodes components, particularly with aging, and also, along the three main axes of bamboo, high disparity in physico-mechanical characteristic is observed (Sekhar and Gulati 1973).

Bamboo is robust; its fiber is two to three times more durable than that of wood. The culms undergo silification and lignification throughout the three to five years it takes them to reach full strength once they have reached full growth. The strength of the culm starts to decline after five to six years (Trujillo, 2007). Height causes the compressive strength to increase while the bending strength falls (Liese, 2015; Espiloy, 1987). According to Bahtiar *et al* 2019, dry bamboo has a higher ratio between the ultimate compression and the mass per unit volume than timber. This was explained by the fact that bamboo has a cellulose content of roughly 55% compared to timber's 50% (Sattar, 1990).

2.1.3 Harvesting and Bamboo Processing

Harvesting of bamboo is much easier than that of timber but more delicate and as such, requires great care. Most products and applications require strength and durability and in the case of bamboo, 4 year old culms are most suited (Oberoi, 2004). This is because this time, the process of lignifications and change in starch content would ensure that the culm is less susceptible to future borer and termite attack thereby preserving its strength (Oberoi, 2004). Depending on species, the diameters of bamboo poles differ. Hatchets (cutlasses) are used in felling and cross-cutting bamboo poles in many places (including Nigeria) but this results in damage to the culm periphery as the bamboo wall is crushed, cracks and splits develop leading to wastage (Oberoi, 2004). As bamboo is prone to splitting due to its nature, the necessity and requirement of hard operated and powered

machinery and tools for harvesting and primary processing of Bamboo cannot be underestimated.

2.1.4 Uses and Importance of Bamboo

The major uses of bamboo, an elaboration on some of the uses is presented as follows in Table 2.1

i. Building Materials

Bamboo is a crucial component of construction. In contemporary constructions, it is utilised for structural members like trusses, kingposts, and purlins.

ii. Panel Made of Bamboo

Bamboo panels were first manufactured in China in the beginning of Victorian period. Over twenty various types of panel products are currently made. Examples of panel products include strip boards, mat boards, fiberboards, particleboards, and medium density fiberboards (Jamatia, 2012).

iii Furniture

In Asia, particularly China, bamboo is utilised to make many types of furniture, like beds, cabinets, tables, and upholstered chairs. The global market for laminated bamboo furniture is expanding quickly, particularly in Asia. (Anerao *et al* 2022)

iv Paper and Pulp

In nations like China and India, bamboo is utilised in pulp and paper production. Paper made from wood are of lesser quality compared to paper made from bamboo. Its brightness and optical qualities do not change over time, unlike paper made from wood, which may change. Morphological characteristic of bamboo fibers made papers produce from it to be of better tear index (Zing and Cheng, 2012).

v. Charcoal

In charcoal production, utilisation of bamboo come into play which acts as an alternative to timber charcoal, also activated bamboo charcoal is utilised for environmental clean-up and moisture absorption (Jamatia, 2012).

vi. Utensils

Utensils such spoons, plates, cups, and serving trays are made from bamboo. Additionally, it is used to make toothpicks.

Table 2.1: Associated Products and Bamboo Industries

Industries	Products
Houses	bamboo-engineered prefabricated homes
Production of pulp and paper	printed materials
Pharmaceuticals and bamboo chemicals	vitamins, amino acids, flavonoids, phenolic acids, polysaccharides, sparse elements, steroids, beverages like beer also ethanol from bamboo, as well as activated carbon.
Energy production	briquettes, biomass feedstock, charcoal, and biofuels.
Textiles	clothing, yarn, socks, and fabrics
Utensils	spoons, cups, serving trays, plates, and forks
Miscellaneous uses in the Industrial sector	extensively utilised in the production of clocks, chains, and fan blades in the electrical, electronic, and communications industries
Farming	pipelines for irrigation and drainage, the use of bamboo leaves in animal feed.

(Sources: Ogunwusi and Onwualu (2013); Okwori and Chado (2013))

2.2 Insects as deteriorating agent

These are some set of insects that feed on wood vascular bundle.

2.2.1 Wood Borers

The phrase "wood boring beetle" refers to a broad group of beetle species and families whose larval or adult stages consume and destroy wood (i.e., are xylophagous). Larval stages of certain insects are commonly known as "woodworms" in the woodworking business. The metallic flat-headed borers, longhorn beetles, bark beetles and weevils are the three groups of wood boring beetles that are the most harmful (Plate 2.3). Some borer species can seriously damage wood, especially when sapwood with high moisture content is employed. These borers are often beetles that tunnel into wood during the larval stage and create a network of galleries that can significantly damage the timber. (Liese *et al,* 2015). Several of the most significant borers are:

- Furniture beetles (Anobiids)
- Pinhole borers
- Powder post beetles (Lyctiids).
- European house borers

Plate 2.3: Front and back view a Wood Borer
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

2.2.2 Carpenter ants (*Camponotus spp.*)

Carpenter ants of several species are capable of destroying the wood in structures like buildings. Their moniker, carpenter ants (Plate 2.4), refers to the way they destroy

wood. Although they don't eat wood like termites do, they nonetheless chew and excrete it to create routes for themselves. Carpenter ants can be identified by their sizes. Carpenter ants also are usually red-with colour ranging from black to red and brown. According to Koehler *et al* 2022 there are over 1,000 species of carpenter ants. Some are non-destructive while some are destructive. In the warmer months, indoor and outdoor spaces sometimes host carpenter ants in both categories. Certain species of carpenter ants can also bite.

Plate 2.4: Carpenter ants that is capable of damaging wood in structures such as buildings
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

2.2.3 Beetles attack

In order to deposit their eggs inside the culm of bamboo, beetles are drawn to the starch in the plant. In the course of beetle maturity, the eggs hatch and larvae feed on the culm till it break through the culm walls to get freed, creating tiny exit holes of different sizes and shapes (Plate 2.5) (about 1mm–6mm diameter). The most prevalent are powder post beetles, which leave 1mm to 2mm exit holes (Koehler *et al* 2022). Green bamboo is highly vulnerable to infestation by beetles and is frequently attacked by them. However, in warm, humid locations with high equilibrium moisture contents, dry bamboo can also be attacked. (Liese *et al*, 2015)

Plate 2.5: Infestation of Bamboo culm by insect with inserted insect
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

2.2.4 Powder post beetle

When it comes to destroying dry seasoned wood, powder post beetles (Plate 2.6) come in second only to termites. They grind the wood till it resembles flour. The growing larvae, which resemble grubs, do harm by boring little, winding tunnels through the wood as they feed. The entire larval life cycle occurs below the surface of the wood, including tunneling.

Infestations are often found after detecting powder and small, circular "shot holes" in the surface of the wood. These are the exit holes that adult beetles made after finishing their development in the wood. Adults that have just emerged mating and laying eggs on or below the surface of unfinished, raw wood. One to five years after the eggs hatch, the small larvae that burrow into the wood become adults. Since some of the beetles are drawn to light, they can occasionally be seen on windowsills or next to damaged wood (Koehler *et al* 2022).

Plate 2.6: Powder post beetle insects that turn wood into a powdery, flour-like substance
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

2.2.5 Termites

The following categories of wood-eating termites are based on how they live and feed (dampwood termites, Ground-dwelling termites, and dry wood termites).

i. Dampwood termites

As the name implies, dampwood termites (Plate 2.7) will only contaminate wood with a lot of moisture. Most damp wood termite species don't need to come into contact with the soil, and their colonies are formed entirely of wood. They continuously consume spring and summer wood against the grain to form connected chambers inside the wood. (Koehler *et al* 2022).

ii. Ground-dwelling termites

Termites that live primarily underground (Plate 2.8) construct their nests in old tree stumps and root systems and regularly venture outside to look for food. As their primary source of food, termites may obliterate everything from building roof structures to flooring made of wood. (Koehler *et al* 2022)

iii. Dry wood termites

Dry-wood termites as their name suggests (shown in Plate 2.9) can invade dry, sound wood. Wooden buildings that are not in direct contact with the earth may get infested. They don't need to be in constant contact with the earth because they may get moisture from the wood they live and eat in. Dry-wood termites have larger galleries and are larger (7–11 mm) than subterranean termites. *Paraneotermes simplicicornis, Incisitermes minor,* and *Marginitermes hubbardi* are examples of dry-wood termites (Koehler *et al* 2022).

Plate 2.7: Damp wood termite; infest wood with high moisture content
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

Plate 2.8: Ground-dwelling termites live primarily underground and construct their nests in old tree
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

Plate 2.9: Dry wood termites are capable of infesting dry, sound wood
(Source: Environmental Bamboo Foundation. Mag: 20) 2017

2.3 Wood Decay Fungi

Fungi attack is the root cause of all lignocellulosic material degrading. Fungi are microscopic creatures that live on and in matter and progressively break down the components of cell walls, causing weakening and degradation. Fungi that cause wood rot feed on the cell walls of wood, causing the wood to deteriorate. Untreated wood will typically decay if it is in direct touch with the ground, cement, or concrete, or if it is exposed to a moisture source like rain, seepage, plumbing leaks, or condensation (Aldo, 2009). Three types of wood-eating fungi are distinguished: brown rot, white rot, and soft rot. The three distinct major chemical elements of wood-lignin, cellulose, and hemicellulose will all be attacked by these various fungi. Wood-degrading fungi break down wood, reducing its strength. The type of fungi found in degrading exercise depend on the type of wood used, and the dimensions of the timber will all affect how much strength is lost.

2.3.1 Brown rot fungi

A dark lignin residue is left behind after brown rot fungi consume cellulose, a component of the wood cell wall. The cellulose is cut into short lengths by a non-enzymatic process as the initial stage of the decay process, leading to a quick early strength loss. A wood infestation may significantly weaken it. Advanced brown rot infestations can be identified by the wood's unusually brown color and tendency to crack across the grain. These fungi typically infect softwood trees including firs, spruces, pines, and spruces (Lebow, 2010).

2.3.2 White rot fungi

These fungi deteriorate the lignin, cellulose, and hemicellulose the three main structural polymers of wood and other lignocellulosic and further metabolize the fragments. Rapidly invading wood cells, the hyphae settle along the lumen walls and secrete an enzyme that depolymerizes hemicellulose, cellulose, and lignin fragments

(Lebow, 2010). Due to the fact that they damage all elements of the cell wall, they are more toxic and damaging than brown rot.

2.3.3 Soft rot fungi

Whether or not there is visible softening of the surface, "soft rot" refers to any incidence of the typical penetration and proliferation of hyphae within the secondary cell walls of wood. It is brought on by a variety of micro-fungi, specifically Ascomycetes and certain *Fungi imperfecti*. All ancient wood that has been exposed to moisture seems to develop soft rot. Since it was discovered that fungi other than Basidiomycetes could deteriorate wood, especially in environments that are not typically conducive to an attack by Basidiomycetes, a lot of effort has gone into comparing the characteristics of soft rot with the two distinctive Basidiomycete rots, "white rot" and "brown rot". The mechanical characteristics of wood that is being attacked by fungi and the potential chemical nature of the degradation process have both received special consideration. Microscopically, soft rot typically differs significantly from both white rot and brown rot (Lebow, 2010).

2.4 Bamboo Preservation and Preservatives

Bamboo is prone to bio-deterioration in its many forms. Fungi, insects, and bacteria are bamboo's principal adversaries and cause damage. By applying preservatives, bamboo can be shielded from these contaminants. The most widely used way of preserving bamboo (for use indoors) is curing it with borax and boric acid since it is efficient. Creosote preservative is capable to increase the service life of *Bambusa vulgaris* to about thirty six years because bamboo culms are treated with Copper chrome arsenate (CCA) (Fazle *et al.*, 2015).

2.4.1 Methods of Applying Wood Preservatives

There are several methods of applying preservatives on bamboo. The treatment approach heavily depends on how the product will be used in the end. There are two main types of bamboo methods of preservation, these are: pressure and non-pressure method.

2.4.2 Pressure Processes

The fundamental idea behind pressure processing is to submerge items in a preservative while applying pressure to drive the preservative into an airtight, steel cylinder or retort. The most efficient technique utilized in the commercial treatment of materials is pressure-induced impregnation with preservatives (Onuorah, 2000). It has a number of benefits, including more uniform and deeper penetration, enabling better control over retention, preconditioning the wood in the chamber, being speedier and more dependable than non-pressure methods, and adhering to code requirements and engineering specifications.

2.4.3 Non-Pressure Processes

When accurate treatment, deep penetration, and high levels of retention are not necessary, non-pressure approaches may be adequate. The type of material to be treated, the amount of moisture in it, the technique and length of treatment, and the preservative employed all affect how effective non-pressure procedures are. There are several ways to add preservatives on bamboo without applying pressure (Onuorah, 2000). Here are some of the techniques

i) Treatments by brushing, spraying, and pouring
ii) Cold soaking
iii) Steeping
iv) Hot and cold bath (thermal process)
v) Double diffusion
vi) Vacuum process

2.7 Bio-preservatives used in bamboo preservation

Bio-preservatives are a diverse group of organic substances derived from herbs and animals that are beneficial for increasing the service life of wood and raising the overall quality of the wood products and decreasing or eliminating the growth of harmful microorganisms (Faruwa *et al.,* 2015). Many studies have been conducted and shown that wood extractive from heartwood are effective as bio-preservatives (Onuorah, 2000). Also

a lot of researches have been carried out on leaves, root, bark and seed of some plant and trees on their efficacy as preservatives against bio-deteriorating agents, which look promising (Nurudeen *et al*., 2012).

Certain plants have been worked upon and discovered as having potentials for bio-preservatives; these include: *Nerium oleander, Neobalanocarpus heimii, Cedar wood oil Cinnamoum camphora, Camphor leaves extract, Valonia Oak, Peppermint Oil, Milicia excelsa, Sternbergia candida, Parthenium argentatu, Cinnamomum camphora, Cryptomeria japonica, Anacardium occidentalel, Aleurites moluccana and Azadirachta indica* Extract (Venmalar and Nagaveni, 2005; Gotaks *et al*., 2007, Kaur *et al*., 2016). Some of the plant extracts offered adequate protection as preservatives on lignocelluloses materials. However, the challenge of fixation still need to be solved (Tor *et al.*, 2007).

2.6 Secondary Plant Metabolites

Basic metabolic activities in plant cells that create chemical substances are secondary metabolites. Albrecht Kossel, who won the 1910 Nobel Prize in physiology or medicine, was the foremost to develop the term "secondary metabolite" (Bourgaud *et al* 2001). He claimed that these compounds are produced from nitrogen metabolism through processes he referred to as "secondary alterations," including deamination. The study of phytochemistry was founded on the increasing recovery of these chemicals in mid-twentieth century, also the discovery chromatography techniques to cause analytical improvements. The chemical structures of secondary plant metabolites which was divided into various kinds fall into several categories, including phenolics, alkaloids, saponins, terpenes, lipids, and carbohydrates.

The presence of one or more phenol groups is inevitable in all (Appendix 1), however straight forward form with aromatic ring to extremely complex polymeric compounds is common to most of them.

2.6.1 Phenolics

According to Bourgaud *et al* (2001), phenolics are the greatest group of secondary metabolites found in trees. About one or more phenol groups is available in all (Appendix

1), however straight forward forms with aromatic ring to extremely complex polymeric compounds is common to all of them. They are abundant in plants and have a considerable impact on the shade, flavour, and aroma of many plants, dish, and beverages. Pharmacologically, a few phenolics are prized for their inimical irritant effects, like quercetin, or for their act against hepatotoxic effects, like sallying. Others have isoflavones properties like genistein and daidzein, while others like naringenin are toxic to insects. Most phenolic compounds are particularly flavonoids, they possess rutin characteristics and are unstable molecules. There categorisation are based on their chemical structure or place of biosynthesis and can as well be divided into simple phenolics, tannins, coumarins, flavonoids, chromones and xanthones, stilbenes, and lignans based on their chemical formations.

2.6.2 Simple phenolics

Eugenol, a phenolic phenylpropane, vanillin, salicylic, ferulic, and caffeic acids are among the varying compounds, depending on their functional group, which might be a hydroxyl, aldehydic, or carboxylic group (phenolic acids). One of the simple phenols with the greatest distribution is hydroquinone, which is found in many herbs as the glycoside arbutin. It is typical for glycosides to develop, and coniferin and other phenolic cinnamic alcohol derivatives serve as precursors to lignin (Evans, 2009).

2.6.3 Tannins

Polyphenols called tannins have the capacity to precipitate protein. For many years, these substances have been utilized to turn unprocessed animal hides into leather. Through the process of cross linking, tannin molecules strengthen the protein's defense against microbial and fungal attack. Condensed tannins and hydrolyzable tannins are the two main categories of tannins. Gallic and hexahydroxydiphenic acids are two phenolic acid molecules that are joined by ester bonds to a main glucose molecule to generate hydrolyzable tannins. Geraniin, which was derived from *Geranium robertianum* (Herb Robert) and *Geranium maculatum* American cranesbill (Catarino *et al* 2017), also tellimagrandins 1 and 2, are ellagitannins that have been discovered in shrubs of medicinal

relevance and for which forms have been clarified (Yi *et al*., 2004) Meadow sweet is a herb that has been separated from pomegranate, oak bark, and Filipendula ulmaria (Evans, 2009).

Condensed tannins, also known as proanthocyanidins, are compounds with oligomeric flavonoid precursor-based structures. These compounds' fusion within flavonoid units, hydroxylation patterns, stereochemistry of the pyran ring's carbons 2, 3, and 4, and the various availability of additional substituents. Few medications, including *Camellia sinensis* tea and *Hamamelis virginiana* leaves and bark, consist condensed and hydrolyzable tannins (Puneet, *et al* 2013).

2.6.4 Coumarins

The lactone of O-hydroxycinnamic acid, benzo-pyrone, is the source of coumarins. The number of isolated natural coumarins is around 1000. More than 30 different families and 150 distinct species of organisms have been reported to have coumarin. The best sources of coumarin are sweet clover, also known as melilot (Melilotus spp.), tonka bean (Dipteryx odorata), and sweet woodruff (Gaium odoratum) (Puneet, *et al* 2013). Common coumarins found in both their loss form and as glycosides in shrubs are *aesculetin, umbelliferone, and scopoletin. Atropa belladonna, Datura stramonium (Solanaceae), Daphne mezereum (Thymeliaceae), Ruta graveolens (Umbelliferae),* and some *Aesculus hippocastanum (Hippocastanaceae)* and *Rosaceae* are inclusive of the plants high in coumarins (Evans, 2009). The most significant biological activity described for coumarins include anti-inflammatory, anticoagulant, anti-cancer, and anti-properties. Alzheimer's (Xu *et al* 2015).

2.6.5 Flavonoids

The majority of phenols that occur in nature are flavonoids. These substances presently number over 2000, with approximately 500 of them occurring in the freestate (Evans, 2009). Flavonoids have chroman rings with an aromatic ring in position 2, 3, or 4 as part of their structural skeleton. According to the degree of oxidation of the core ring, there are several classes (ring C) of flavonoids in sub-divisions. The prevalent of them are

flavones, flavonols, as well as anthocyanins. Flavones and their families are normally yellow (Latin flavus, yellow). Although they are naturally available, they are more abundant in vascular plants and as well as in juvenile fiber's cell sap. The *Polygonaceae, Rutaceae, Leguminosae, Umbelliferae*, and *Compositae* all have a lot of them. Recent research has shown the health consequences of drugs consisting flavonoids such as *Glycyrrhiza glabra* (liquorice root), *Chmaemelum nobile* (Roman chamomile) and *Ginkgo biloba* (gingko).

2.6.6 Chromones and xanthones

Though not of significant medicinal significance, a few of these compounds eugenin from the clove shrub and khellin from mustard seeds derivatives of benzo-pyrone are worth mentioning (Evans, 2009). The active ingredients in the fruits of Ammi visnaga, furanochromones, are more complicated. Xanthones are primarily available in the *Gentianaceae* and *Guttiferae*, although they are also rarely available in the *Moraceae* and *Polygalaceae* families of plants. The mountaineers of Malawi and its neighbours utilise *Polygala nyikensis* to cure a variety of epidermis conditions with a fungus origin. Due to the presence of xanthones, the plant's root was recently demonstrated to exert its antifungal effect (Susana et al., 2011).

2.6.7 Stilbenes

A heterogeneous collection of plant species contains a tiny but widely dispersed category of secondary metabolites known as stilbenes, which are primarily found as heartwood components. They play a significant role in the heartwood of trees belonging to the *Pinus (Pinaceae), Eucalyptus (Myrtaceae), and Madura genera (Moraceae)*. The trendiest stilbene in universe is resveratrol, a para-hydroxylated substance. Resveratrol is available in *Picea, Pinus*, the *Fabaceae*, the *Myrtaceae*, and the *Vitaceae* and exhibits estrogen-like activity (Gehm, *et al* 1997).

2.6.8 Lignans

The fusion of 2 molecules of a phenylpropene derivative of Asteraceae (e.g., *Achillea lingulata* (Puneet, *et al* 2013), *Pinaceae* e.g., *Cedrus deodara* (Sharma *et al.*, 2008) and *Rutaceae* e.g., *Fagara heitzii* (Mbaze, *et al.*, 2009) form lignans. There are 4 major types which occur: dibenzylbutane derivatives, dibenzylbutryolactones (lignanolides or derivatives of butanolide), monoepoxy lignans or derivatives of tetrahydrofuran and bisepoxylignans or derivatives of 3,7-dioxabicyclo(3.3.0)-octane. Antimicrobial and antifungal are exhibited by most of these compounds (Seigler, 1995), while others exhibits anti-tumour nature, the like of wikstromal, matairesinol and dibenzyl butyrolactol from *Cedrus deodara* (Mbaze, *et al.*, 2009). Lignans are two units compounds that come together formed the fusion of two molecules of a phenylpropene outgrowth and have been noticed in the *Asteraceae, Pinaceae*, and *Rutaceae* families of plants, including *Achillea lingulata*, *Cedrus deodara* and *Fagara heitzii* (Mbaze, *et al*., 2009). The four main subtypes of lignans are: monoepoxy lignans, or derivatives of tetrahydrofuran; dibenzylbutane derivatives; dibenzylbutryolactones, or derivatives of 3,7-dioxabicyclo(3.3.0)-octane. Wikstromal, matairesinol, and dibenzyl butyrolactol from *Cedrus deodara* were among the substances that demonstrated antibacterial and antifungal properties, while others demonstrated cytotoxic properties (Mbaze, *et al.*, 2009).

2.6.9 Alkaloids

Numerous alkaloids are highly poisonous to animals and can be deadly when consume. Many (e.g., nicotine and anabasine) alkaloids are the main elements in production of poison against insects and pests (Mbaze, *et al.*, 2009). Alkaloids are organic substances that include a heterocyclic ring with not less one nitrogen atom. Alkaloids are not similar unit of chemicals from any point of view, whether chemical, biochemical, or physiological, which makes them difficult to describe. For this reason, no single description can truly apply to all alkaloids, however it is clear that they all contain nitrogen substances. Alkaloids can be sub-divided into many types based on their fundamental chemical makeup, including acridones, aromatics, carbolines, ephedra, ergots, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolines, quinozolines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrrolidines,

(Tadeusz, 2015). Many alkaloids are poisonous enough to kill animals when consumed. Several are employed as insecticides, including nicotine and anabasine (Mbaze, *et al.*, 2009).

2.6.10 Saponins

Saponins are the fusion of a steroid (steroidal saponins) or triterpenoid (triterpenoidal saponins) and a carbohydrate unit (a monosaccharide or oligosaccharide chain) (Figures 8 and 9). Pentoses, hexoses, or uronic acids and many more, make up the sugar units. Lowering surface tension by saponins is achieved because of the hydrophobic-hydrophilic asymmetry and they have soap like nature. The possibility to isolate these compounds from plants leaves, stems, roots, bulbs, flowers, and fruits, however, there is high concentration of these compounds in the roots of species like, *Digitalis purpurea* (foxglove), *Dioscorea villosa* (wild yam), *Eleutherococcus senticosus* (*Siberian ginseng*), *Gentiana lutea* and many more. Research as shown that about 500 plants from not less than 90 different families has high saponins content (Tadeusz, 2015).

2.6.11 Terpenes

The most numerous and varied class of secondary chemicals found in plants are terpenes. The word "turpentine," is a derivative of an ancient French word ter(e)bintb, which denotes "resin," gives rise to the name "terpene." Chemically, 5-carbon isoprene units fuse together in numerous manners to form terpenes (Tadeusz, 2015). Prefixes in terpene naming determines there different categorise based on molecule make up in term of number isoprene units.

2.6.12 Hemiterpenes

Hemiterpene are just one isoprene unit. The oxygen-containing by-product of isoprene, like angelic acid obtained from Angelica archangelica and isovaleric acid from *Vaccinium myrtillus*, are thought to be the only hemiterpenoids (Tadeusz, 2015).

2.6.13 Monoterpenes

Chemically, they are described by the formula $C_{10}H_{16}$ and 2 isoprene units make up (Figure 10). They are crucial elements of volatile or essential oils derived from plants. Many essential oil source plant families contain monoterpene, such plant families as *Lamiaceae, Pinaceae, Rutaceae, and A paceae*. Volatile secretions like geraniol are sparsely available in a lot of plants, this is practically universal. Monoterpenes has subtypes, which are; unsaturated hydrocarbons (such as limonene), alcohols (such as linalool), alcohol esters (such as linalyl acetate), aldehydes (such as citronellal), and ketones (e.g., Carvone). Numerous common medical applications exist for monoterpenes and other volatile terpenes. Camphor and menthol are two substances that are utilised as analgesics, anti-inflammatories, and counterirritants. Monoterpenes have a long history of usage as anthelmintics. Numerous monoterpene glycosides seem to have a vasodilating impact on the femoral vascular bed and coronary arteries (Sharma, *et al* 2008).

2.6.14 Sesquiterpenes

Their chemical formula is $C_{15}H_{24}$ and they have three isoprene units (Appendix 11). Sesquiterpenes come in more than 200 different structural kinds and thousands of these chemicals are recognised, depending on their biogenetic origin and are of three major classes which depend on structural activities: acyclic (such as farnesol), monocyclic (such as bisabolol), and bicyclic (e.g., caryophyllene). The antibacterial, antifungal, and antiprotozoan properties of many sesquiterpene lactones are demonstrated. At quantities comparable to metronidazole, an antiamoebic medication, sesquiterpenes from Vernonia colorata inhibit *Entamoeba histolytica*. Flowers from Arnica montana have cardiotonic qualities because of helenialin and a group of similar chemicals. In clinical settings, the rhizome of *Atractylodis macrocephala* (Asteraceae) acts as a diuretic, analgesic, and anti-inflammatory. Eudesma-4(14)-7(1 l)-dien-8-one and atractylenolide I are two active substances that are associated with the activity. Because sesquiterpenes are present, therefore other similar related medicinal shrubs are also used for the same aim (Kisiriko *et al* 2021).

2.6.15 Diterpenes

Chemically, formula is $C_{20}H_{32}$ and 4 isoprene units in a diterpene (Figure 12). Acyclic and macrocyclic diterpenes are different types of chemicals. Additionally, macrocyclic diterpenes are divided into groups based on how many ring systems they include. Diterpenes can combine 5- and 7-membered ring structures or 6-membered ring structures. Large number of diterpenes possesses multiple ring structures. These exist as esters that are side replacements. The pharmacological effects of diterpenes, like those of all terpene groups, include analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic, and antiprotozoal actions. A few diterpenes from the Ericaceae family plant *Kalmia latifolia* exhibit antifeedant effects on gipsy moths. The diterpenoid acids known as gibberellins, which were first separated from the fungus of the genus Gibberella, available also in vascular plants, have a notable impact on seedling growth (Evans, 2009).

2.6.16 Sesterterpenes

It is hard to come by terpenes that contain twenty five carbons and five isoprene units (the sester- prefix means ½ to 3, i.e. 2 ½), as shown in the group of other sizes. Geranyl farnesol, which was discovered from the seed oils of *Camellia sasanqua (sasanqua) and Camellia japonica (camellia),* family *Theaceae*, is an example of a sesterterpenoid. Mouse leukemic M1 cells shown cytotoxic action for geranyl farnesol (Kisiriko *et al* 2021).

2.6.17 Triterpenes

The description of triterpene chemically consists of 6 isoprene units with formula $C_{30}H_{48}$ (Appendix 13). It is the main component of shark liver oil. The linear triterpene squalene, is produced by the diminishing fusion of 2 different molecules of farnesyl pyrophosphate. More than 4000 triterpenoids have been identified, and terpenes make up a sizeable fraction of all plants' lipid compounds. In both plants and animals, these substances act as precursors to steroids. Steroids and triterpenes can both be seen in natural form, as glycosides, or in various combinations. Triterpene and steroid structures can be split into around 40 primary categories. Boswellia carterii's oleo-gum resin

contains two triterpenes called -boswellic acids that have been identified for their anti-inflammatory and anti-rheumatic properties (Culioli *et al.*, 2003).

The quassinoids identified from Quassia amara are one class of chemicals that exhibit a variety of intriguing biological activity. These are the byproducts of triterpene breakdown and rearrangement. Quassia is element in the production of poison against insect, a bitter tonic, and an enema to get rid of thread worms.

2.6.18 Lipids

A class of naturally occurring compounds known as lipids includes phospholipids, fixed oils, waxes, essential oils, sterols, and fat-soluble vitamins including A, D, E, and K. Lipids has the ability to biologically function as essential structural elements to every biological membranes, to keep energy, energise for cellular processes, as well as to being vitamins and hormones (Fahy *et al*., 2009, Subramaniam, *et al.*, 2011). Although, they are recognised as the fundamental plant metabolites, current research has shown that several of the phytochemicals in this class have pharmacological functions.

2.6.19 Fixed oils

Molecular aliphatic of high value with elongated-chain fatty acids, such as oleic, palmitic, and stearic acids, are what make up fixed oils. Esterified glycerol is fatty acids. Fixed oils are much richer in liquid polyunsaturated glycerides like glycerin oleate than fats, which are greater in solid glycerides like glycerin stearate (Fahy *et al*., 2009). *Linum usitatissimum*, which belongs to the family Linaceae, is the source of flax, linseed, and its oil. Some fixed oils contain polyunsaturated fatty acids that inhibit the excretion of lipid peroxidation products, making them strong rutin and pain reducing agents. Ability to lower the danger associated with atherosclerosis and cardiovascular disease. (Richard *et al*, 2008).

2.6.20 Waxes

Wax is a particular kind of lipoidal substance composed mostly of long aliphatic chains, some of which may contain functional groups. Similar to fundamental and the

advance long-chain alcohols, which normally seen as esters, hydroxyl groups may be inclusive. The remaining consists of unsaturated bonds, amide or ketonic functional systems, carboxylic or aromatic functional groups, or aromatic functional systems. In contrast, synthetic waxes are created from long-chain hydrocarbons (paraffins or alkanes) devoid of functional groups. They resemble fixed oils and fats because they are fatty acid esters, however they differ from glycerin in that they do not contain glycerin as their alcohol (Masotti *et al*, 2003).

2.6.21 Essential oils

Complex volatile mixes with components that have an aromatic scent of low molecular weight is refer to essential oil. Despite having up to 60 different components, essential oil known for having 2 or 3 primary constituents at relatively high concentrations (20–70%) contrast to other constituents that are only present in trace levels. The two main components of *Origanum compactum* essential oil are Carvacrol (30%) and thymol (27%), for instance. With a concentration of up to 68%, linalol is the primary constituent in *Coriandrum sativum* essential oil. Other examples include *Cinnamomum camphora* essential oil, which contains 1,8-cineole (50%) as main component, *Mentha piperita* essential oil, which contains menthol (59%) and menthone (19%) as main constituents, and *Artemisia herba-alba* essential oil, which consist of - and -thuyone (57%) and camphor (24%) as main constituents. Typically, these key elements dictate how the essential oils' biological qualities behave (Pichersky *et al*, 2006). They serve a wide range of vital medicinal purposes, including antiseptic, antibacterial, analgesic, sedative, anti-inflammatory, spasmolytic, and local anaesthetic treatments. Along with embalming and food preservation, they are also utilised as perfumes (Masotti *et al.*, 2003).

2.6.22 Carbohydrates

All living things on our planet have carbohydrates. Carbohydrates are the precursor to every phytochemicals and, by addition, every animal biochemicals because they are the earliest product of photosynthesis. More than any other sort of natural molecule, carbohydrates are found in nature. Carbohydrates are incorporated in large

amount of secondary metabolites through glycosidation linkages, but all the same they are primary metabolites. The by-product of mucilages and gums are polymers of simple sugars and uronic acids (Pichersky *et al*, 2006).

Practically all plants and some microorganisms produce mucilage, a viscous, sticky material that thickens plant membranes for defence. Additionally, it facilitates food and water storage as well as seed germination. An exopolysaccharide and a polar glycoprotein make up its chemical composition.

2.3.1 *Datura metel*

Datura metel L is from the Solanaceae family of 9 species vespertine flowering plants. The following are example of the family; Thorn Apple, Pricklyburr, Moonflower, Hell's Bells, Devil's Weed, Devil's Cucumber, and Devil's Trumpet (from large trumpet-shaped flowers). They are shrubs, leafy and short-lived perennials with maximum height of two metres. They are found throughout the temperate and tropical regions of the whole world and this reason why knowing their exact natural distribution in the whole universe is difficult, the species diversity occurs mostly in the United States and Mexico. The alternating leaves have a lobed or serrated border and measure 10–20 cm long by 5–18 cm wide. The blooms are trumpet-shaped, erect or spreading, and range in colour from white through yellow, pink, and pastel purple. They are not pendulous like those of the closely related Brugmansiae family. When ripe, the fruit splits open to release the numerous seeds. The fruit is a spiny capsule that is 4–10 cm long and 2–6 cm wide. The seeds are easily dispersed throughout pastures, fields, and even areas of wasteland.

2.3.2 *Euphorbia hirta*

The plant genus Euphorbia and family Euphorbiaceae include *Euphorbia hirta.* It is a reddish or purplish, annual, hairy plant with a thin stem and several branches growing from the base to the top. The opposing, elliptic-oblong to oblong-lanceolate, acute or subacute, dark green above; pale underside, 1 to 2.5 cm long, purple-blotched in the centre, and toothed at the edge, leaves are opposite. E. hirta leaf methanolic extract has antifungal and antibacterial properties. Warm leaves that have been mashed with turmeric

and coconut oil are applied to itchy soles. Similar to surma, the latex of E. hirta is applied to the lower eyelids to treat eye ulcers. The root exudate has nematicidal effects on meloidogyne incognita juveniles (CIRS, 2005). Leaf extracts in methanol exhibit antifungal and antibacterial properties. Warming and applying the crushed leaves to itchy soles with coconut oil and turmeric. In the same way as surma is used to treat eye sores, E. hirta's latex is applied to the lower eyelids. Meloidogyne incognita juveniles are susceptible to the nematicidal effects of the root exudate (CIRS, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sourcing for plants, collection and processing

Datura metel and *Euphorbia hirta* were obtained at Onipe (latitude: 7.2^0 North, Longitude: 3.88333^0 East) and Ejioku (latitude: 7.4720^0 North, Longitude: 4.0759^0 East), Ibadan, Oyo state respectively, identification and registration of the plants were done by a taxonomist at the Forest Herbarium, Ibadan, Forestry Research Institute of Nigeria (FRIN). The plants were registered as *Datura metel* (Gegemu) - No FHI: 110211 and *Euphorbia hirta* (Emi-Ile) - No FHI: 110210. The harvested shrub plants were rinsed with tap water and spread under a cool dry shade for four weeks (Plates 3.1 and 3.2). After drying, the two plants were separately packed in a doubled layer rice bags and stored in a cool dry place in the laboratory. The air-dried plants (Plate 3.3) where later pulverized using a milling machine in FRIN, Soil Laboratory (Plate 3.4).

Plate 3.1: *Datura metel* Sited at Onipe environs, Ibadan

Plate 3.2: *Euphorbia hirta* Sited at Ejioku environs, Ibadan

Plate 3.3: Air drying of *Euphorbia hirta* stock under a shed

Plate 3.4: Milling machine in FRIN Soil Laboratory

3.2 Extract Generation from *Datura metel* (Gegemu: No FHI: 110211) and *Euphorbia hirta* (Emi-ile: No FHI: 110210) - extract yield determination.

The pulverized *Datura metel* (Gegemu: No FHI: 110211) were transferred into the soxhlet apparatus made up of the extractors, round bottom flasks, sitting on electrical heaters (Plate 3.5) and containing solvent (Ethanol). Extraction was done in accordance with the T2 0403-76 standard (Kazemi *et al.*, 2006) in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan.

The pulverized *Detura metel* and solvent (ethanol) in the extractors were placed on the heater for three days at 78°C to ensure complete extraction. The solvent extractive mixture was collected in 500 ml round bottom flask extractors; the flasks were connected to the rotary evaporator (Plate 3.6) and the temperature switch was regulated to 78°C, while the rotary evaporator was regulated to 7 revolutions per minute (rpm). The solvent was gathered and kept in the 100 ml round bottom flask the generated oily *Datura metel* extract were left in the 500 ml round bottom flask. The exercise was repeated for pulverized *Euphorbia hirta.*

In determining the extract yield of *Datura metel* and *Euphorbia hirta,* 20g of the pulverized samples of each plant was loaded in the single loader sohxlet extractor apparatus, the weight of the generated extracts using distil water, Toluene, N-exane and absolute ethanol as solvent at different times were done, which gave the extract yield weight of the eight generated extracts. Amount of extracts collected was determined by

$$\% \text{ Extract } = \frac{\text{Volume Collected}}{Weight of Initial Material} X\ 100 \qquad \ldots \qquad 3.1$$

Plate 3.5: A complete set of multiple Soxhlet Extractor Apparatus

3.3 Extracts Storage

The extractives from four different solvents were differently loaded in a rotator evaporator (Plate 3.6) in FRIN's central laboratory for concentration. Thereafter the obtained extracts (Plate 3.7) were stored inside a freezer in the laboratory. Thirty (30) grams of *D. metel* extracts was dissolved in prepared 5,000 ml distil water inside 10 litre transparent kegs and was thoroughly shaken to ensure extract is well dissolved in distil water. By that exercise, 100% concentration of *D. metel* natural preservative of 5,000 ml quantity was realised. The formulation was carried out at four different concentration levels: 25% concentration (500 ml of each extractive diluted with 1500 ml of distilled water) in a 5 litre transparent keg; 50% concentration (1000 ml of each diluted extractive in 1000 ml of distilled water), 75% concentration (1500 ml of each extractive diluted with 500 ml of distilled water) while 100% concentration (undiluted), see Table 3.1. The same procedure was repeated for the second extractive (Plates 3.8 and 3.9) according to AOAC (2000).

Table 3.1: Formation ratio of Bio-Preservation of four levels of Concentrations

S/N	Conc. Level %	100% Conc. Bio-Preservative Vol. (ml.)	Distil Water Vol. (ml)	Total Vol. (ml) of Bio-Preservative Formulated
1	25	1,500	500	2000
2	50	1000	1000	2000
3	75	500	1500	2000
4	100	0	2000	2000

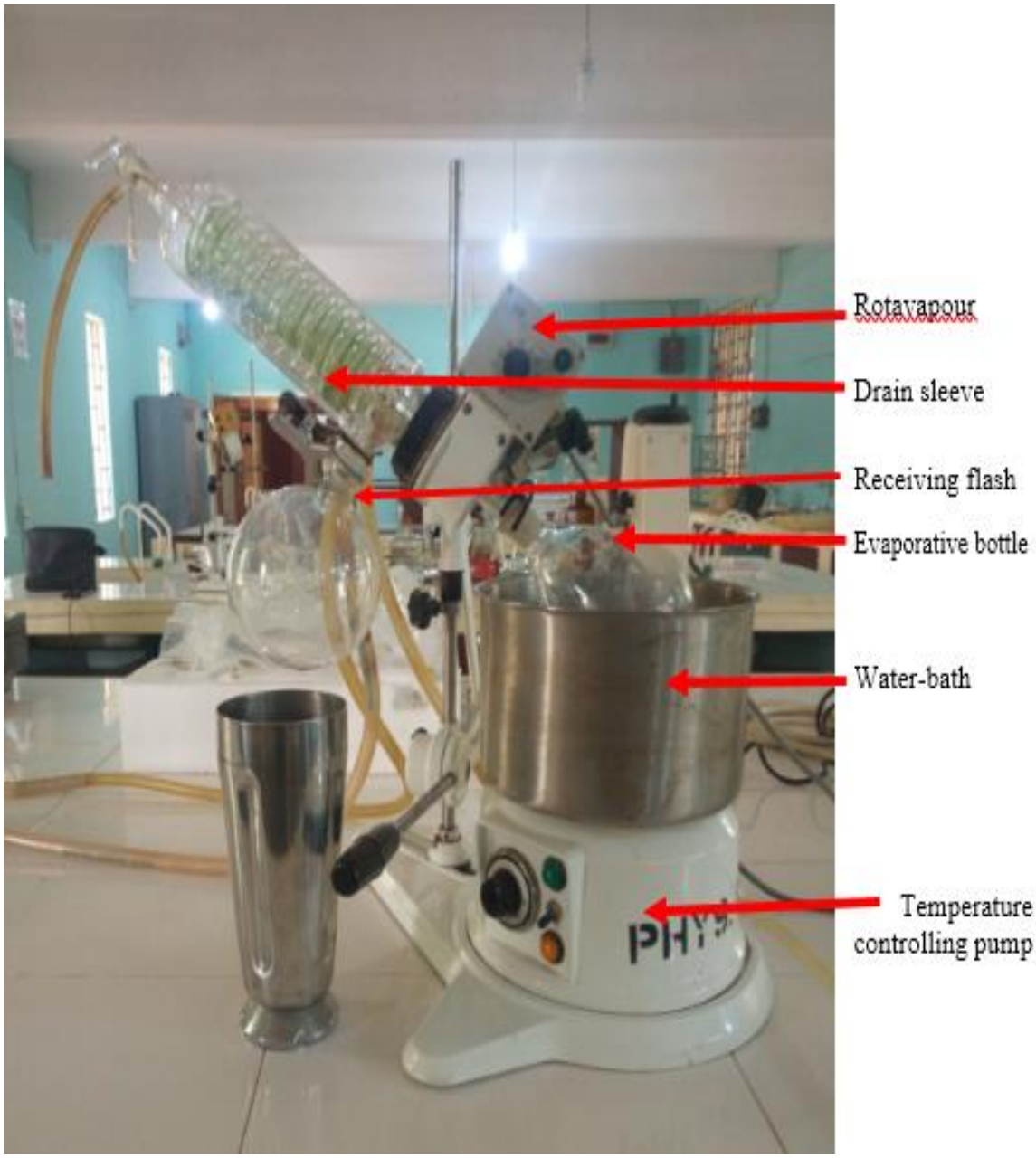

Plate 3.6: Rotary evaporator used to concentrate extractive to obtain extract

Plate 3.7: Extracts of *Datura metel* and *Euphorbia hirta.*

Plate 3.8: Formulated preservatives from *Datura metel* extract using water as effluent

Plate 3.9: Formulated preservatives from *Euphorbia hirta* extract using water as effluent

3.4 Screening for Metabolites and Chemical Constituents

3.4.1 Phytochemical screening (Qualitative and Quantitative)

Primary metabolites such as saponins, flavonoids and tannins are reported to deactivate the activities of biodeteriorating agents such as fungi and termites (Congyi Zhu and Jianxion Li; 2019, Waller, G. R. and Yamasaki, K; 1995), these metabolites were screened for, in the extracts obtained from *D. metel* and *E. hirta* both qualitatively and quantitatively. The procedures were carried out on the pastry sample according to AOAC (2000) according to Harborne (1993) was conducted at the Biomedicinal Research Centre (BMRC) in Forestry Research Institute of Nigeria, Jericho, Ibadan.

3.4.2 Saponins

The pulverised plant sample (20 g) was placed into a conical flask. The sample was then mixed with 100 ml of 20% ethanol (C_2H_5OH). The conical flask containing sample was placed in a water bath, cooked to 55°C for 4 hours while being constantly stirred. The generated mixture was then filtered. Thereafter the gathered extractive was mixed with 200 ml of ethyl alcohol. The produced extractive was then concentrated and reduced to 40ml while the conical flask containing the generated extractive was placed on a water bath at 90°C. The produced concentrate was then kept in a 250 ml separating funnel, thereafter, 20 ml of diethyl ether (CH_3CH_2) was added, and the generated mixture was intensely agitated after which the diethyl ether layer was noticed and poured away, and the concentration procedure was repeated after recovering the aqueous layer. Addition of 60 ml of n-butanol (C_4H_9OH) into the by product and then, the extractive was twice washed with 10 ml of 5% sodium chloride (NaCl). Thereafter, evaporation procedure took place before the sample was dried in an oven to a consistent weight with the residual solution. The amount of extract gathered was calculated using

$$\text{Flavonoid}\left[\frac{\text{Calculate of saponins content}}{amount of saponins\left(\frac{mg}{g}\right)}\right] = \frac{weight of residue}{weigh of sample} \quad \ldots. \quad 3.2$$

3.4.3 Flavonoid

Qualitative and quantitative for flavonoids was screened by going through the following steps using gas chromatography and mass spectrometry.. About 1 g of the plant extract, cooked together with 10 ml of ethanol. Thereafter, 2 drops of ferric chloride was added to 5 ml of the extractive generated (Plate 3.10).

It was observed immediately that a dusty green colour showed up, after 5 ml of the extractive was again set aside and a drop of dilute NaOH was added also drops of concentrated HCl were ran down the side of the tube. A reddish colouration was observed which confirmed the presence of flavonoids.

The determination of the quantitative processes for the total flavonoids was done following these AOAC (2000) procedures. The complicated flavonoid-aluminum formation technique was used this purpose. The extractive (1 mg/ml) was added with 0.5 ml of 2% AlCl-ethanol solution. The by product was allowed to settle down for about an hour as this will allow yellow colour to show up, confirming the presence of flavonoids. The absorbance at 420 nm of the extractive was carried out with the use of a UV-VIS spectrophotometer. Quercetin equivalent (mg/g) of total flavonoid content was determined with the help the equation derived from the curve Y=0.255x, R =0.981 2, where x is the absorbance and Y is the quercetin equivalent.

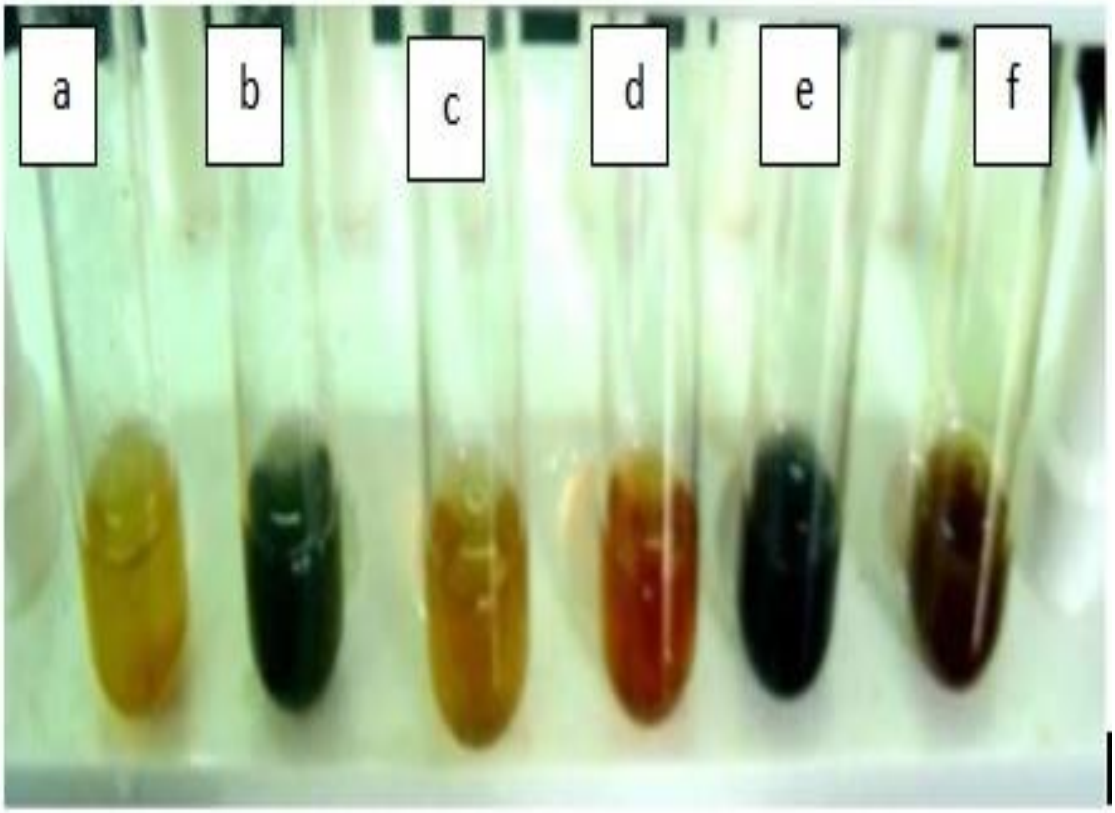

Plate 3.10: A Set of Test Tubes for Phytochemical Screening test

3.4.4 Tannins

Creation of vanillin-HCl reagent was carried out with the measure of equal volumes of 8% HCl and 1% vanillin in methanol were mix for that purpose. A conical flask was filled with 0.2 g of the powdered substance, and the reagent was mixed right before use. Next, 10 ml of 1% concentrated HCl in methanol was added. The conical flask was well sealed and seriously shaken for 20 minutes, after which the contents were centrifuged at 2,500 rpm for an additional five minutes. A test tube containing 5 ml of the vanillin-HCl reagent was pipetted with 1 ml of the supernatant. Following 20 minutes of incubation at 30°C, the spectrophotometer read an absorbance of 450 nm (AOAC 2000). The result was expressed as catechin equivalent value using a standard curve, as shown below.

$$\text{Tannins}(\%) = C\ X\ \frac{20}{200} \quad \ldots \quad 3.3$$

Where, C stands for concentration correspond to the official density

In view of the findings above, it was necessary to know the chemical constituent of the two extracts, by this decision, gas chromatography screening was needful.

3.5 Gas Chromatography Screening

The chemical constituents present in the samples were via gas chromatography analysis (Plate 3.11) (Agilent 19091s-433) with a mass spectrometer and HP-5MS 5% phenyl methyl siloxane column (30 m x 0.25 mm x 0.25 µm) at Basel Convention Coordinating Centre for Training & Technology Transfer for the African Region, University of Ibadan. As soon as the sample (1 µL) was sent into the column, the oven's temperature was regulated to 90 °C and held for 0 seconds, and then ramped up to 180 °C for 10 seconds. By comparing the spectra of the chemicals with those in the NIST library, the carrier gas helium (purity 99.999%) was used to confirm the products.

Plate 3.11: Gas Chromatography Image
(Basel Convention Coordinating Centre for Training and Technology Transfer for the African Region, UI)

3.6 Bamboo Conversion

The five culms of bamboo (*Bambusa vulgaris*) replicates were harvested at bamboo natural forest of Forestry Research Institute of Nigeria, Jericho, Ibadan (North East Local Government Area). Six hundred (600) bamboo experimental samples were generated from the five (5) different culms with samples fetched along the length (Figure 3.1) of each culm (Base, Middle 1, Middle 2, and Top). The test samples were all of dimension 60 x 20 x 4 mm for both termite and fungi test (Onilude, 2016) as shown in Plate 3.12 and Figure 3.2.

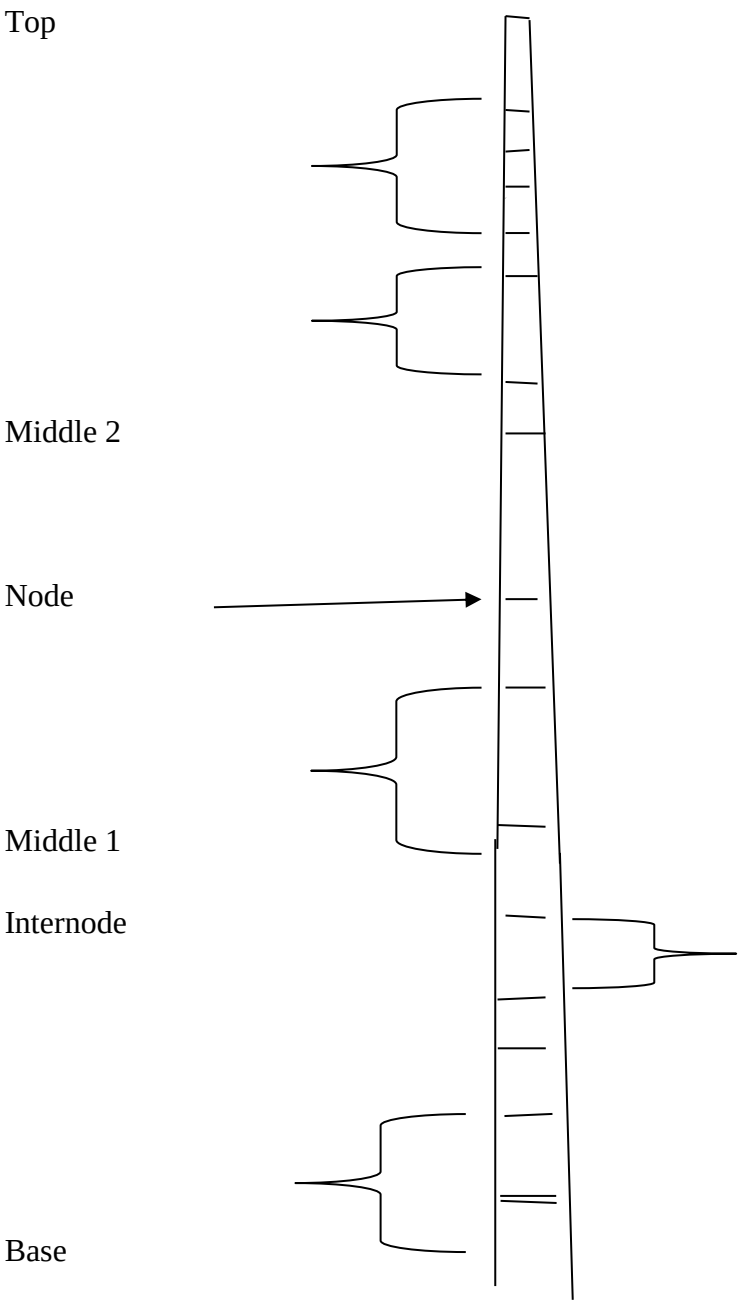

Figure 3.1: Schematic diagram of sampling technique from bamboo culm

Plate 3.12: Experimental bamboo samples generated for termite and fungi decay tests

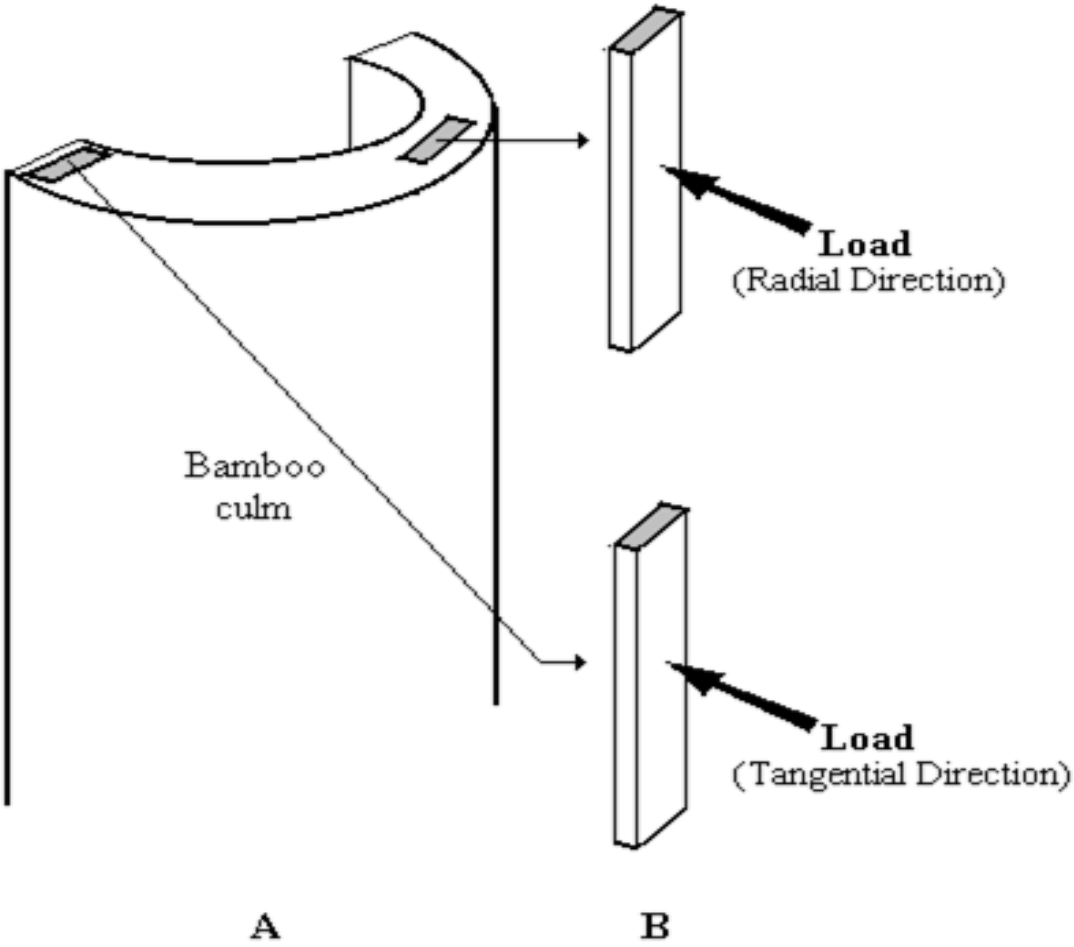

Figure 3.2: Test samples generated are of 60 x 20 x 4 mm

(Sources: nIG/mAP/ .com (2014))

3.7 Determination of Moisture content

The bamboo splits were weighed with the use of sensitive weighing instrument. The initial weights of every sample were noted (T1). All the samples were kept in oven with the temperature regulated to 70±2°C to dry till a constant weight of each one of them are achieved, thereafter, the weight of all of the sample were noted (T2) to determine the moisture content using oven dry method following the procedure of ASTM D 4442-84 (1986), according to equation 3.4.

$$\mathrm{MC}(\%) = \frac{\mathrm{T1} - \mathrm{T2}}{\mathrm{T2}} X\ 100 \qquad \ldots \qquad 3.4$$

Where: M C % = Moisture content, T1 = Weight of sample before oven-drying (g), T2 = Weight of sample after oven-drying (g)

3.8 Preservative Treatment

The six hundred (600) bamboo split samples prepared for these decay tests were oven dried, after which, four hundred and eighty (480) pieces were selected for treatment by immersion for 72 hours in eight different transparent covered bowls containing formulated preservatives from two different plant extracts (*Datural metel* and *Euphorbia hirta*) at four different concentration levels, (25%, 50%, 75%, 100%) for each plant extract. Sixty samples were treated in each formulation (20 each for termite, brown rot, and white rot), keeping 15 samples as control for each concentration level (5 each, for termite, brown rot and for white rot) (Nurudeen, *et. al.*, 2012). The whole treated bamboo experimental samples (480 pieces), were all conditioned (air drying) for a week after which, the weight were noted (T3).

3.8.1 Determination of percentage absorption

The weights of bamboo samples after treatment and conditioned were recorded to determine level of absorption rate. It was calculated using the formula below according to Adetogun, 2009.

$$\text{Absorption rate} = \frac{\text{Condition Weight} - \text{Oven dry Weight}}{\text{Oven dry Weight}} \text{ x } 100 \quad \ldots. \quad 3.6$$

3.9 Test for determining efficacy of preservative treatment on bamboo

Termite and Fungi test (brown rot fungus, *Sclerotium rolfsil*' and white rot fungus, *Pleurotus florida***)** were carried out. In determining the preservative potential of the two formulated bio-preservatives at difference concentration level(s), this required to impact treatment permanence on bamboo experimental samples and evaluation of the efficacy of different concentrations of the formulated bio-preservatives in relation to their resistant to termite and fungi using ASTM procedure. The treated (480) bamboo experimental samples using bio-preservatives formulated from *D. metel* and *E. hirta* at concentration, 25%, 50%, 75% and 100% of extracts for each and the control (120) bamboo experimental samples were sub-divided into A, B and C groups at 200 bamboo samples per group (160 treated and 40 control). Group A was exposed to termite (*Macrotermis bellicosus*) attack in a timber graveyard test for 12 months while groups B and C were inoculated with brown rot (*Sclerotium rolfsii*) and white rot (*Pleurotus florida*) fungi respectively for 24 weeks, using weight loss WL to evaluate and assess efficacy of the bio-preservatives used for the treatment of the bamboo experimental samples.

3.9.1 Termite Experiment

Experimental design for termite test was a completely random 2x4x4 factorial experiment that was replicated five times.

Factor A: Two (2) plants *(Datura metel* and *Euphorbia hirta*)

Factor B: Extract concentration (4) (25%, 50%, 75% and 100%)

Factor C: Four (4) sampling height (first internodes, three internodes interval to the fourth one.

Total population of test samples is 160, by the above experimental design. The mathematical models;

Model for 2 x 4 x 4 factorial experiment in a Completely Randomized Design (CRD)

Total = 32 treatment combinations x 5replicates = 160samples

$$Yijlk = \mu \times Ai \times Bj \times Cl \times ABij \times ACil \times BCjl \times ABCijl \times \sum kijl \quad \ldots\ldots. (i)$$

Where, Yijlk = general mean, μ = general mean, Ai = effect of factor A, Bj = effect of factor B, Cl = effect of factor C, ABij = effect of interaction between factors A and B, ACil = effect of interaction between factors A and C, BCjl = effect of interaction between factors B and C, ABCijl = effect of interaction among factors A, B and C, ∑kijl = experimental error, I = level of factor A (2 levels), j = level of factor B (4 levels), l = level of factor C (4 levels), k = number of observation.

(Source: Akindele, 2004)

3.9.2 Experimental Design for Fungi Test

Fungi test experimental design was a completely random 2 x 2 x 4 x 4 factorial experiment that was replicated five times.

Factors A: 2 plants (*Datura metel and Euphorbia hirta*).

Factor B: 2 bio Agents (Brown rot fungi-lignin, White rot fungi-cellulose)

Factor C: 4 extract concentration levels (25%, 50%, 75%, 100%)

Factor D: 4 sampling height (first internodes, three internodes interval to the fourth one).

Total population of test samples is 320, by the above experimental design. The mathematical models;

Model for 2 x 2 x 4 x 4 factorial experiment in a Completely Randomized Design (CRD)

Total = 64 treatment combinations x 5replicates = 320samples

$$Yijlk = \mu \times Ai \times Bj \times Cl \times Dn \times ABij \times ACil \times ADin \times BCjl \times BDjn \times CDln \times$$

$$ABCijl \times ABDijn \times ACDiln \times ABCDijln \times \sum kijln \quad \ldots \quad (ii)$$

Where, Yijlk = general mean, μ = general mean, Ai = effect of factor A, Bj = effect of factor B, Cl = effect of factor C, Dn = effect of factor D, ABij = effect of interaction between factors A and B, ACil = effect of interaction between factors A and C, ADin = effect of interaction between factors A and D, BCjl = effect of interaction between factors B and C, BDjn = effect of interaction between factors B and D, CDln = effect of interaction between factors C and D, ABCijl = effect of interaction among factors A, B and C, ABDij = effect of interaction among factors A, B and D, ACDiln = effect of interaction among factors A, C and D, ABCDijln = effect of interaction among factors A, B, C and D, ∑kijln = experimental error, i = level of factor A (2 levels), j = level of factor B (2 levels), l = level of factor C (4 levels), n = level of factor D (4 levels), k = number of observation

(Source: Akindele, 2004)

3.9.3 Grave yard Test for Termite (*Macrotermis bellicosus*)

The treated one hundred and sixty bamboo experimental samples and forty untreated (control) samples were planted in termite infested grave yard (Plate 3.13) at the

Forestry Research Institute of Nigeria's Department of Forest Products Development and Utilization, Jericho, Ibadan. These samples were left in the grave yard for twelve months and observation on weight losses was made at the end of the twelfth month using ASTM D 1413-76 (1986) test procedures.

The planted bamboos were taken out of the grave yard after the exposure period, and the soil was thoroughly cleaned off the bamboo surfaces. All the bamboo samples (200) were conditioned by storing them under room temperature for 24 hours. Thereafter, they were all weighed again and Percentage weight losses were calculated from the conditioned weight, using

$$Weightloss(\%) = \frac{T3-T4}{T3} X\ 100 \qquad \ldots \qquad 3.7$$

Note, T3 = Weight of conditioned treated and control bamboo sample before planting on grave yard; T4 = Weight of conditioned sample after removal from grave yard


TIMBER
GRAVE YARD

Plate 3.13: Timber Grave Yards site in FRIN

3.9.4 Decay Test

The three hundred and sixty (360) preservative treated bamboo samples (180 samples, each for brown rot, *Sclerotium rolfsii'* and white rot *'Pleurotus florida*') were prepared for fungi test (Plate 3.14) while fungi spawn were obtained (Plates 3.15 and 3.16). Inoculation was carried out in the laboratory (Plate 3.17) inside transparent bowls (Plate 3.18) with cover along with 40 untreated (control) bamboo samples. The inoculation plates (Plates 3.19 and Plate 3.20) were covered and incubated at normal temperature in the room (28 ± 2°C) for 24 weeks (Plate 3.21 and Plate 3.22). The weight losses were recorded at the end of the inoculation exercises using ASTMD1413-76 (1986) procedures for solid wood. After the inoculation exercises, the bamboo samples were taken and myceliums were carefully brushed off the samples surfaces and conditioned. Percentage weight losses of the samples using the formula below were calculated after conditioning for a week and weight of samples taken (T4)

$$Weightloss(\%) = \frac{T3-T4}{T3} X\ 100 \qquad \ldots. \qquad 3.7$$

Note: T3 = Weight of treated and conditioned sample; T4 = Weight of conditioned of sample after exposure to fungi

Plate 3.14: Bamboo samples being prepared for fungal (white or brown rot) inoculation

Plate 3.15: Brown rot '*Sclerotium rolfsii*' fungi spawn

Plate 3.16: White rot '*Pleurotus florida*'Fungi spawn

Plate 3.17: Inoculation exercise being carried out in the Laboratory

Plate 3.18: Inoculated Bamboo samples in the inoculation box

Plate 3.19: Brown rot fungus growing on bamboo samples treated with *D. metel* bioextract

Plate 3.20: White rot fungus growing on bamboo samples treated with *E. hirta* bioextract

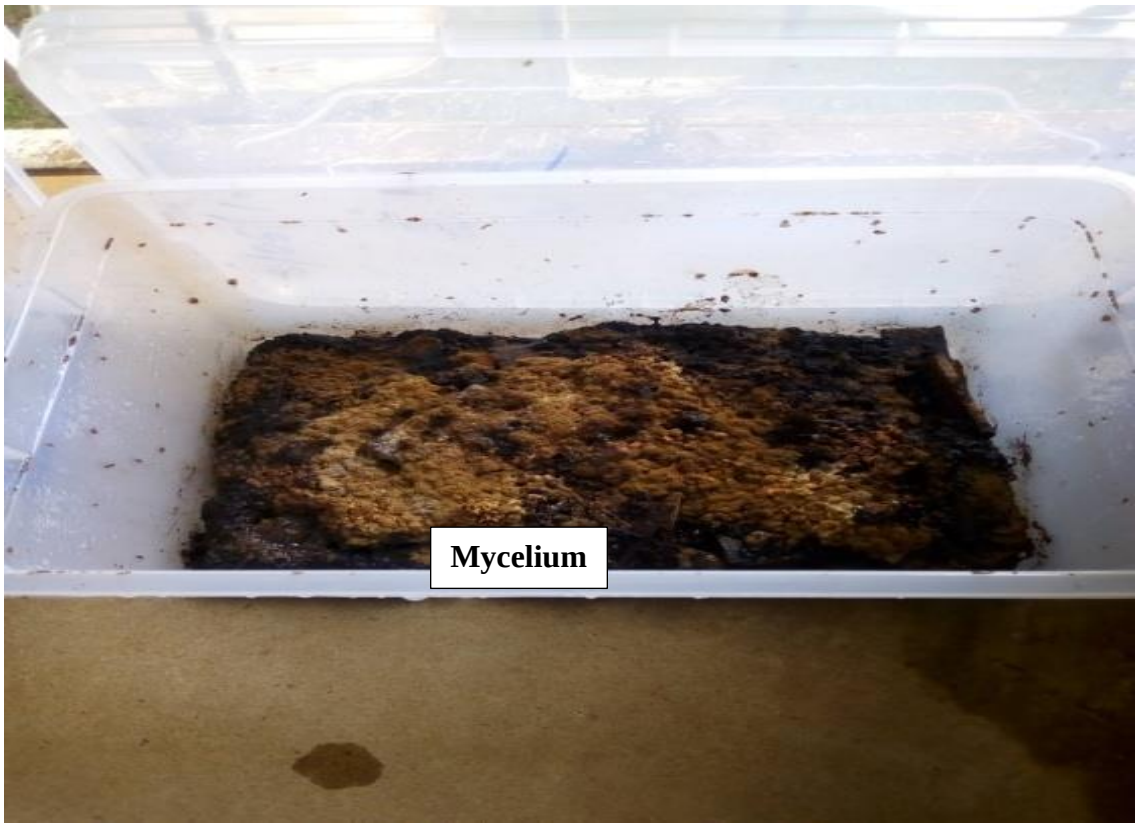

Plate 3.21: Site of bamboo samples inoculated with brown rot fungi after 24 weeks observation

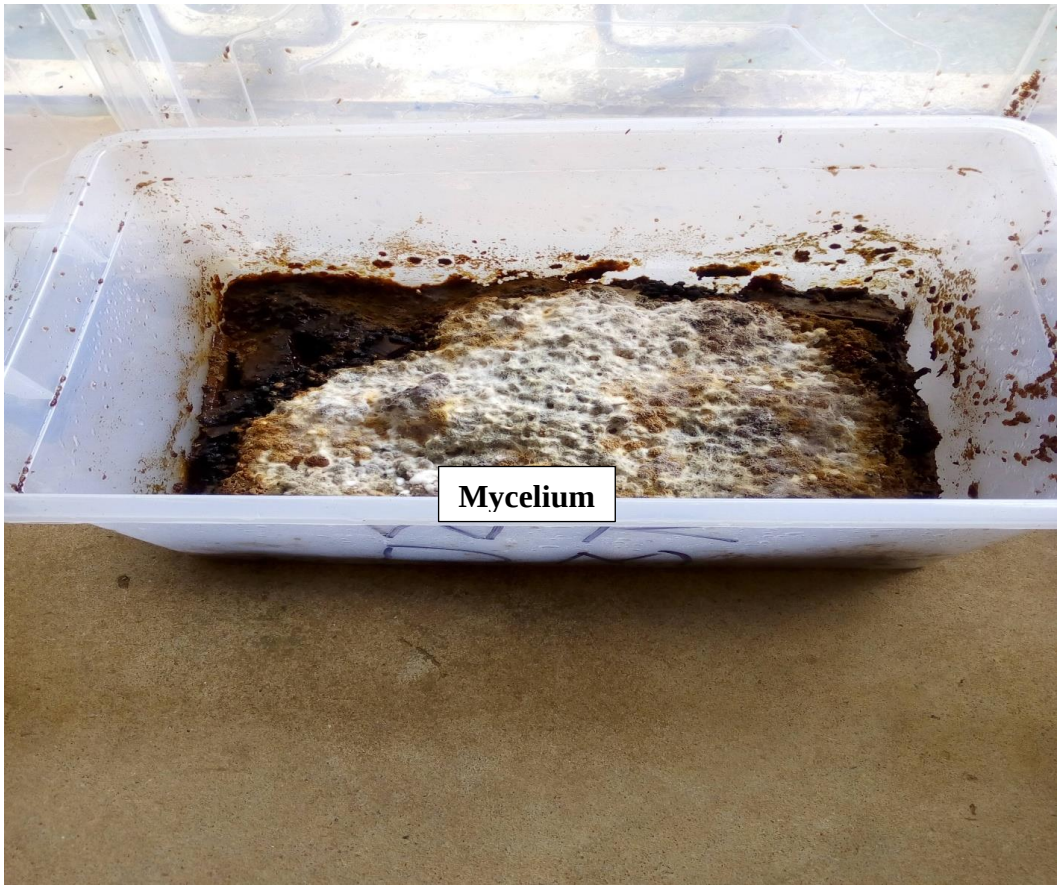

Plate 3.22: Site of bamboo samples inoculated with white rot fungi after 24 weeks observation

3.10 Laminate Production

Production of bamboo strips were conducted at the University of Ibadan's Department of Forest Production and Products. To create straight pieces, the culm sections were chopped from the base to a length of 2 metres. Each piece was cut into the appropriate number of axial slices, which were then air-dried beneath a shed. To create bamboo strips with a thickness of 0.6 cm, the inner and outer faces of each slice were machined off. Using a metal spatula, a top bond of 12 g was placed to each surface of the bamboo strip. The strips were then stacked to form bamboo laminates of 2.5 cm thickness and pressed for three (3) days (Plates 3.23 and Plate 3.24).

3.10.1 Treatment of the bamboo laminated board (BLB)

The laminated bamboo board was later planed and forty (40) Dimensional samples measuring 20 mm by 20 mm by 20 mm (Plate 3.25) were generated using a narrow band saw machine, out of which thirty two (32) pieces were treated by immersion for three (3) days in eight (8) different formulated preservatives (four (4) samples in each preservative) after which the treated samples alongside with the eight (8) samples untreated ones (control), making forty (40) samples in all. With the use of moisture meter, the moisture content of all these samples were taken after conditioning for three days and recorded.

Plate 3.23: Prepared bamboo strips with the thicknessing machine

Plate 3.24: Laminated bamboo strips using top bond glue as binder

Plate 3.25: Shear strength samples processed from laminated bamboo boards

3.10.3 Mechanical test to evaluate the impact of treatment on glue line of bamboo laminate board.

Experimental design for Shear strength test was a 2x5 factorial experiment in a completely Randomized Design replicated 5 times

Factor A: Two (2) plants *(Datura metel and Euphorbia hirta*)

Factor B: 4 Extract concentration (25%, 50%, 75%, 100%) and control.

Total population of test samples is 50, by the above experimental design. The mathematical models;

Total = 8 treatment combinations x 5 replicates = 50 samples

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \Sigma_{kij} \quad \ldots\ldots\ldots\ldots \quad \text{(iii)}$$

Where: Y_{ijk} = individual observation, μ = General mean, A_i = effect of factor A, B_j = effect of factor B, AB_{ij} = effect of interaction between factor A and B, Σ_{kij} = experimental error, i = level of factor A, j = level of factor B, k = Number of observation

(Egbewole, *et al*., (2021)

3.10.4 Shear Strength Parallel to Grain

This test was carried out in accordance to ASTM D143-52 of 1997. A total of thirty-two (32) treated and eight (8) untreated samples were subjected to load on Universal Testing Machine (Plate 3.26) in Wood Mechanics Laboratory, Forestry Research Institute of Nigeria's Department of Forest Products Development and Utilization, Jericho, Ibadan, to determine shear resistance perpendicular to grain on the treated and control laminated board cubes to evaluate the effect on the two formulated preservatives on the glue line. All through the experiment, the load was exerted at a rate of 0.6 mm/min, thereafter the failure of the samples (Plate 3.27) at a certain load which was recorded, after which the ultimate shear stress was calculated using the following equation

$$\text{Shear strength (N/mm}^2\text{)} = \frac{P}{bd} \quad \ldots\ldots \quad 3.9$$

Note, P = Maximum load, b = breath, d = width

Plate 3.26: Shear Strength Parallel to Grain test of samples on Universal Testing Machine

Plate 3.27: Failed laminated bamboo samples after shear test

3.11 Fixing of Natural Preservative Chemical Compounds in Bamboo

Beaker of 1000 ml by volume was filled with 500 ml distilled water (Plate 3.28), after which twenty bio-preservative treated bamboo samples (at the formulated

concentrations) were dropped into it and left for 24 hours (Plates 3.29 to 3.33), these samples were removed and then conditioned thereafter, visual observations were made on the filtrate distilled water in the beaker (Olorunisola, 2019).

Plate 3.28: Beaker Containing 500 ml distilled water

Plate 3.29: Twenty pieces of treated bamboo samples dropped in beaker containing distilled water

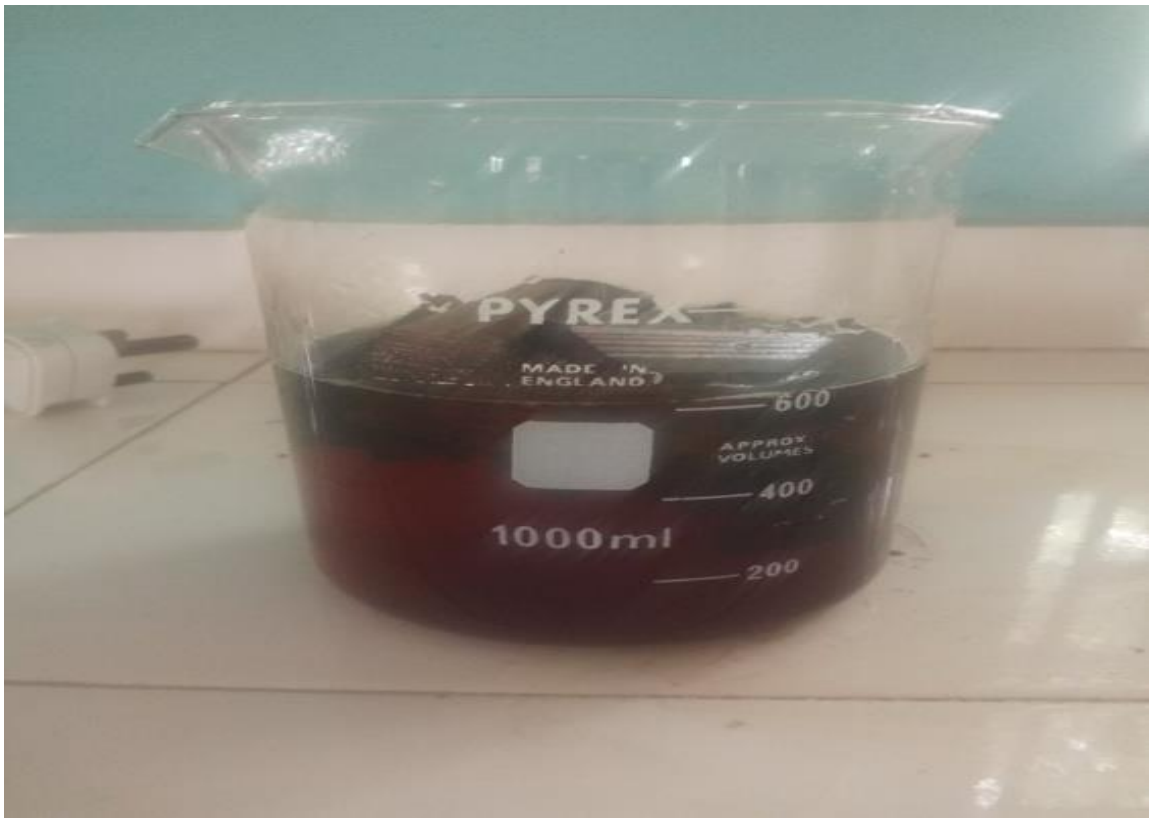

Plate 3.30: Stained distilled water after soaking treated bamboo samples for 24 hrs

Plate 3.31: Filtrate left after removal of treated bamboo samples

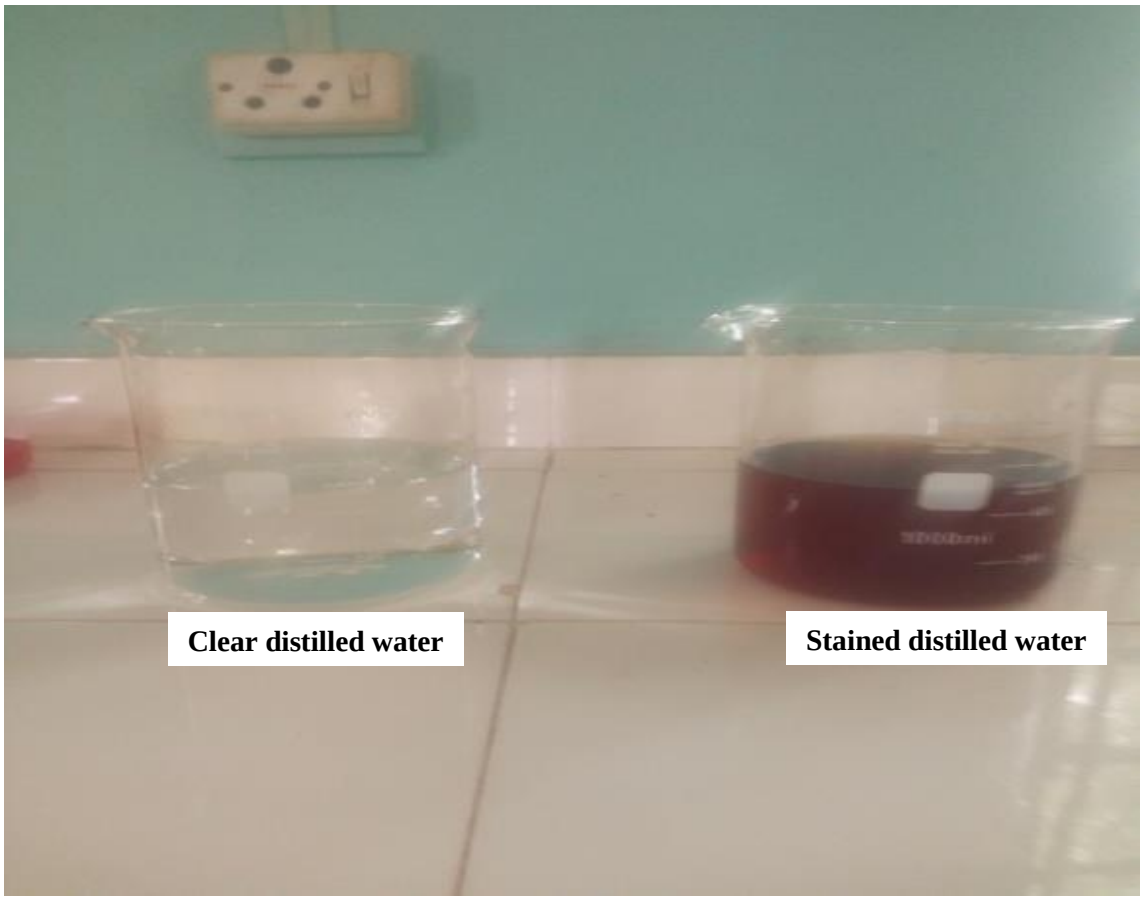

Plate 3.32: Visual observation of solvents

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results on Extraction Yield (mass of extract)

Extraction yield based on weight of extracts generated from plants was presented in Table 4.1. Using distilled water as solvent, the extract yields from *Datura metel* and *Euphorbia hirta* were 2.12 g and 1.69 g, respectively. The extraction yield (using absolute ethanol as solvent), were 1.85 g and 1.00 g for *Datura metel* and *Euphorbia hirta*, respectively. This indicated that, in terms of yield, distilled water generated more extracts from the selected plants compared with absolute ethanol. It may mean that polarity difference of the solvents influenced extraction yield. This is in conformity with Bajwa *et al.* (2008) who discovered that extraction yield from plants using distilled water was more than that of ethanol. More so, it was observed that extraction yield of *D. metel* was more than *E. hirta*. This could be attributed to concentration of sample/solvent and solubility of the components in *D. metel* which resulted to higher extraction yield in distilled water and ethanol.

4.2 Phytochemical screening and Characterisation of the Extracts

The qualitative and quantitative phytochemical of extracts obtained from the two plants: *Datura metel* and *Euphorbia hirta* were presented in Table 4.1. Results showed the presence of saponins, flavonoids and tannins. The weights of phytochemicals were 0.28 mg/g, 0.12 mg/g and 0.04 mg/g (in *D. metel*); and 0.31 mg/g, 0.11 mg/g and 0.027 mg/g (in *E.hirta*) for saponins, flavonoids and tannins, respectively.

Saponins were the most predominant in terms of weight (0.28 mg/g and 0.31 mg/g) in *D. metel* and *E. hirta*, respectively; this was followed by flavonoids and tannins. The weight of flavonoids was more in *D. metel* (0.12 mg/g) than *E. hirta,* (0.11 mg/g).

Table 4.1: Extract yield and quantitative phytochemical Screening

Plant extract	Solvent	Yield (g)	Metabolites (g)		
			Saponins	Flavonoids	Tannins
D. metel	Distilled water	2.12	0.28	0.12	0.04
	Absolute ethanol	1.85			
E. hirta	Distilled water	1.69	0.31	0.11	0.027
	Absolute ethanol	1.00			

This may mean that there is higher concentration of flavonoids in *D. metel* than *E. hirta*. Furthermore, tannins in *D. metel* (0.04 mg/g) were more than in *E. hirta* (0.027 mg/g). This may mean that there is higher concentration of tannins in *D. metel*.

The higher percentage composition of phytochemicals in *D. metel* could have been due to concentration of phytochemicals in the plant during extraction and type of solvents used.

The presence of saponins, flavonoids and tannins was as found by (Catarino *et al* 2017) and (Yi *et al* 2004), this implied that the plant extracts have potentials to deactivate fungi activities. Hence, they can be used as bio-preservatives against fungi.

Visual inspection of the bio-preservatives formulated from *Datura metel* and *Euphorbia hirta* extracts revealed two different colour shades. The bio-extracts from *Datura metel* were in reddish green colour, while that of *Euphorbia hirta* were in leafy green colour. The concentration levels (25%, 50%, 75% and 100%) of the formulations influenced the colouration of the extracts. The higher the concentration of the two categories, the darker the colour, in addition, extracts generated from *D. metel* are less oily and more soluble in distilled water when compared to that of *E. hirta* extract.

4.3 Chemical Compounds present in bio-extracts based on gas chromatography screening

4.3.1 Chemical Compounds (percentage composition) present in *Datura metel*

Table 4.2 revealed that chemical compounds present in *Datura metel* include Dodecanoic acid, ethyl ester (10.89%), Tetra decanoic acid, ethyl ester (15.67%), Cyclohexanol, 2-(methylaminomethyl)-trans (4.11%), Bicyclo [3.1.1] heptane, 2,6,6,-trimethyl-1α,2β,5α (29.82%), 2-Pentadecanone, 6,10,14- trimethyl (18.29%), 2-6, 17-Octadecadien-1-01 acetate (7.04%), 1,2 – Dihexylcyclo propene (11.69%), 3,3 – Dimethyl -4- methyl amino – butan-2- one (2.49%).

The highest (29.82%) and least (2.49%) percentage composition of the chemical compound were found in 2,6,6,-trimethyl, 3,3-Dimethyl-4-methyl amino-butan-2-one, and bicyclo [3.1.1] heptane, respectively. Consequently, in line with submission of Yi *et al*., (2004), heterocylic substances (such as 2,6,6,-trimethyl Bicyclo [3.1.1] heptane) are being explored as potential agrochemicals such as antifugal and antitermite agents.

Table 4.2: Chemical compounds in *Datura metel*

S/N	Retention time	Relative area	Compound Name	% Composition
1	23.780	9.60	Dodecanoic acid, ethyl ester	10.89
2	30.915	13.80	Tetra decanoic acid, ethyl ester	15.67
3	32.137	2.51	Cyclohexanol, 2-(methylaminomethyl)-trans	4.11
4	32.635	30.34	Bicyclo [3.1.1] heplane, 2,6,6,-trimethyl(1α,2β,5α)	29.82
5	32.952	20.13	2-Pentadecanone, 6,10,14- trimethyl	18.29
6	33.729	7.16	2-6, 17-Octadecadien-1-01 acetate	7.04
7	34.563	14.57	1,2 – Dihexyl cyclo propene	11.69
8	35.229	1.90	3,3 – Dimethyl -4- methyl amino – butan-2- one	2.49

4.3.2 Chemical Compounds (percentage composition) present in *E. hirta*

Table 4.3 revealed that *Euphorbia hirta* extract contains the following compounds: Bicyclo [3.1.1] heptane, 2,6,6 –trimethyl-1α,2β,5α (52.60%), 1-Alanine, N-(1-oxopentyl) methyl ester (5.97)%, 3-Azabicyclo (3-2-2) nonane (17.73%), 11-Tetradecyn-1-01 acetate

(23.70%). *E. hirta* heterocylic substances, therefore they are being explored as as antifugal and antitermite agents according to Yi *et al*., (2004).

Table 4.3: Chemical compounds in *Euphorbia hirta*

S/N	Retention time	Relative area	Compound Name	% Composition
1	32.629	66.72	Bicyclo [3.1.1] heptane, 2,6,6–trimethyl (1α,2β,5α)	52.60
2	32.946	3.94	1-Alanine, N-(1-oxopentyl), methyl ester	5.97
3	33.722	13.74	3-Azabicyclo (3-2-2) nonane	17.73
4	34.544	15.61	11-Tetradecyn-1-01 acetate	23.70

4.4 Bio-deterioration Test

4.4.1 Efficacy of bio-extract as preservative against Termite (*Macrotermis bellicosus*)

Table 4.4 revealed that weight loss of *Datura metel* treated samples ranged from 0.62±0.29 to 1.75±0.57 for 25% and control, respectively. It was observed that, resistance of *Datura metel* treated samples to termite attack was higher at all levels of concentrations than control. Hence, highest weight loss (1.75±0.57) g was recorded for control while treated samples had lower values (ranging from 0.62±0.29 to 0.83±0.50) g. Furthermore, WL of samples obtained from different sampling height revealed that middle 2 had least (0.82±0.54) g WL while Top had the highest value (1.04±0.60) g. This may mean that top portion of bamboo culm did not retain as much bio-extracts as middle. Hence, its resistance against termite attack was lower- leading to higher weight loss. More so, top portion may be more naturally perishable than middle. Hence, higher preservative concentration may be required for its treatment to be as effective as middle.

For *Euphorbia hirta*-treated samples, the WL ranged from 0.77±0.40 g to 1.74±0.57 g for 75% and Control, respectively. The highest WL (1.74±0.57) g recorded for control signified that it was the most attacked by termites. This means that control was the least resistant to attack. Hence, it was the most susceptible to biodegradation on exposure to termite attack.

Result revealed that WL ranged from 1.00±0.58 g to 1.04±0.75 g for Base and Middle 1, respectively. Furthermore, WL of samples treated with *Datura metel* was lower than that of *Euphorbia hirta*-treated samples. This implied that *Datura metel* samples had more resistance to termite attacked compared to *Euphorbia hirta*-treated samples.

The result presented on Tables 4.5 and 4.6 revealed that extract (treatment effect) was significant on weight loss. That is, there was significant difference in WL between *D. metel and E. hirta*-treated samples. More so, there was significant difference in WL between preservative concentrations. However, effect of sampling height was not significant on WL at $p \leq 0.05$. The result of preservative concentration on weight loss of bamboo after exposure to termite attack was shown in Table 4.4. This effect shows that WL of bamboo was the same at all levels of concentration, but different to that of control.

It was observed that the WL of control (1.75) g was different from treated samples (0.79; 0.86; 0.77 and 0.96) g for

Table 4.4: Weight loss of samples subjected to termite attack (graveyard test) (g)

Extracts	Sampling height	Concentration					Pooled mean
		25%	50%	75%	100%	Control	
DM	Base	0.38±0.11^{a}	0.90±0.32^{c}	0.62±0.35^{c}	0.64±0.34^{c}	1.64±0.56^{ab}	0.87±0.61
	Middle 1	0.41±0.19^{ab}	0.70±0.21^{ab}	0.50±0.19^{bc}	0.96±0.70^{b}	1.93±0.42^{b}	0.82±0.54
	Middle 2	0.78±0.27^{c}	0.69±0.50^{a}	0.83±0.34^{b}	1.04±0.51^{a}	2.16±0.75^{a}	0.98±0.57
	Top	0.92±0.10^{d}	0.67±0.50^{a}	1.04±0.52^{a}	0.67±0.38^{c}	1.42±0.42^{c}	1.04±0.60
	Pooled mean	**0.62±0.29**	**0.74±0.38**	**0.75±0.40**	**0.83±0.50**	**1.75±0.57**	**0.93±0.58**
EH	Base	1.28±0.32^{a}	1.18±0.65^{a}	0.80±0.24^{b}	0.75±0.35^{c}	1.64±0.60^{ab}	1.00±0.58
	Middle 1	0.68±0.51^{b}	1.48±1.25^{a}	0.91±0.45^{a}	1.34±0.67^{a}	1.93±0.57^{b}	1.04±0.72
	Middle 2	0.83±0.64^{b}	0.54±0.66^{c}	0.52±0.27^{c}	1.37±1.07^{a}	2.16±0.75^{a}	1.04±0.75
	Top	1.06±0.70^{a}	0.75±0.50^{b}	0.91±0.61^{a}	0.90±0.48^{b}	1.42±0.45^{c}	1.02±0.57
	Pooled mean	**0.79±0.48**	**0.86±0.65**	**0.77±0.40**	**0.96±0.61**	**1.74±0.57**	**1.03±0.66**

Note: mean values with the same alphabets on the same column are not significantly different ($p \leq 0.05$)

Table 4.5: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Sampling Height (SH) and Extract types of bamboo after exposure to termite attack

S/N	SH	Dm (g)	Eh (g)
1	Base	0.87 ± 0.61^{b}	1.00 ± 0.58^{a}
2	Middle 1	0.82 ± 0.54^{b}	1.04 ± 0.72^{a}
3	Middle 2	0.98 ± 0.57^{ab}	1.04 ± 0.75^{a}
4	Top	1.04 ± 0.60^{a}	1.02 ± 0.57^{a}

Note: mean values with the same alphabets are not significantly different ($p\leq0.05$)

Table 4.6: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Conc. Level % and Extract types of bamboo after exposure to termite attack

S/N	Conc. Level	Dm (g)	Eh (g)
1	25	0.62 0.29^{d}	0.79±0.48^{bc}
2	50	0.74 0.38^{c}	0.86±0.65^{bc}
3	75	0.75 0.40^{c}	0.77±0.40^{c}
4	100	0.83 0.50^{bc}	0.96±0.61^{b}
5	Control	1.75 0.57^{a}	1.74±0.57^{a}

Note: mean values with the same alphabets are not significantly different ($p \leq 0.05$)

25%, 50%, 75% and 100% concentration, respectively. All the concentration levels had the same WL. The implication was the percentage of termite consumption on control (untreated samples) was significantly different to that of the treated bamboo samples at all levels of concentrations.

4.4.2 Efficacy of bio-extract as preservative against Brown rot fungus '*Sclerotium rolfsil*')

Table 4.7 revealed that weight loss of *D. metel* bio-preservatives samples (after exposure to brown rot infestation) ranged from 1.22±0.42 g to 1.89±0.33 g for 75% to control, respectively.

Based on sampling height variation, the least (1.25±0.40) and highest (1.89±0.33) WL were recorded at top and base, respectively. It was observed that the values increased from top to base. This implied that resistance to brown rot infestation was least at the base and highest at the top.

For *Euphorbia hirta* bio-preservatives based treated samples the WL values ranged from 0.76±0.58 to 1.86±0.69. More so, the WL ranged from 0.49±0.39 to 1.55±0.60 for base and Middle 2, respectively. Result revealed that, unlike in white rot infestation, WL of *D. metel*-treated samples was more than that of *Euphorbia hirta*-treated samples (at all levels of concentration). The implication was that, *Euphorbia hirta* extract preserved samples of *B. vulgaris* against brown rot infestation more than *D. metel* extract at all levels of concentration. Hence, higher percentages of WL were recorded for *D. metel* after exposure to brown rot fungi. The ANOVA for Weight loss of samples exposed to brown rot infestation (Tables 4.7) revealed that all treatment effects and their interactions (except Conc. * SH and EXT* Conc * SH) were significant on WL. Furthermore, results (Table 4.9) revealed that WL of control was different while WL of samples treated at other concentration levels were the same. This implied that, resistance of control (untreated

samples) to brown rot fungi was not the same compared to treated samples (at all concentration levels).

Tables 4.8 and 4.10 (Effect of sampling height on weight loss) revealed that WL of

Table 4.7: Weight loss of samples exposed to brown rot infestation (g)

Extracts	Sampling height	Concentration					Pooled mean
		25%	50%	75%	100%	Control	
DM	Base	1.45±0.18^{a}	1.54±0.38^{a}	1.44±0.48^{a}	1.54±0.26^{a}	2.04±0.36^{a}	1.60±0.39
	Middle 1	1.53±0.34^{a}	1.25±0.29^{b}	1.46±0.26^{a}	1.27±0.30^{b}	2.05±0.29^{a}	1.51±0.40
	Middle 2	1.19±0.20^{b}	1.38±0.18^{c}	1.03±0.34^{b}	1.47±0.30^{a}	1.82±0.09^{b}	1.38±0.35
	Top	1.22±0.26^{bc}	1.25±0.36^{b}	0.96±0.39^{c}	1.17±0.35^{bc}	1.65±0.41^{bc}	1.25±0.40
	Pooled mean	**1.35±0.27**	**1.36±0.31**	**1.22±0.42**	**1.36±032**	**1.89±0.33**	**1.44±0.40**
EH	Base	0.46±0.25^{a}	0.27±0.22^{a}	0.32±0.20^{a}	0.35±0.22^{a}	1.05±0.46^{a}	0.49±0.39
	Middle 1	0.80±0.20^{b}	0.51±0.16^{b}	0.46±0.18^{a}	0.29±0.19^{b}	1.66±0.65^{b}	0.74±0.58
	Middle 2	1.40±0.21^{c}	1.12±0.12^{c}	1.41±0.34^{b}	1.30±0.61^{c}	2.54±0.12^{c}	1.55±0.60
	Top	1.33±0.34^{c}	1.48±0.32^{cd}	1.40±0.33^{b}	1.09±0.37^{c}	2.20±0.21^{cd}	1.50±0.48
	Pooled mean	**1.00±0.46**	**0.85±0.53**	**0.90±0.58**	**0.76±0.58**	**1.86±0.69**	**1.07±0.69**

Note: mean values with the same alphabets on the same column are not significantly different ($p \leq 0.05$)

Table 4.8: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Sampling Height (SH) and Extract types of bamboo after exposure to Brown Rot

S/N	SH	Dm (g)	Eh (g)
1	Base	1.60±0.39	0.49±0.39
2	Middle 1	1.51±0.40	0.74±0.58
3	Middle 2	1.38±0.35	1.55±0.60
4	Top	1.25±0.40	1.50±0.48

Note: mean values with the same alphabets are not significantly different ($p \leq 0.05$)

Table 4.9: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Conc. Level % and Extract types of bamboo after exposure to Brown Rot

S/N	Conc. Level	Dm (g)	Eh (g)
1	25	1.35±0.27	1.00±0.46
2	50	1.36±0.31	0.85±0.53
3	75	1.22±0.42	0.90±0.58
4	100	1.36±032	0.76±0.58
5	Control	1.89±0.33	1.86±0.69

Note: mean values with the same alphabets are not significantly different ($p \leq 0.05$)

Table 4.10: Result of sampling height on weight loss (g)

Sampling height	Mean values
Base	1.05[a]
Middle 1	1.13[a]
Middle 2	1.47[b]
Top	1.38[b]

Note: means with identical letters do not differ significantly

base and Middle 1 were not the same with that of Middle 2 and top. The result implied that, after exposure to brown rot infestation, WL of samples obtained from base and middle 1 were not the same as that obtained at middle 2 and top. This could be attributed to variation in lignocellulosic composition such as vascular bundles and parenchyma of *B. vulgaris* along axial direction (Shukla, *et al.*, 1988).

4.4.3 Efficacy of bio-extract as preservative to combat White rot fungus '*Pleurotus florida*')

The weight loss recorded of samples exposed to white rot infestation (Table 4.11) revealed that WL of *D. metel*-treated samples ranged from 0.16±0.08 to 0.81±0.51. However, least (0.25±0.13) and highest (0.52±0.55) WL were recorded for Top and Base respectively. For *Euphorbia hirta*-treated samples, least (0.28±0.24) and highest (0.71±0.33) WL were recorded for 75% and control, respectively. Result also revealed that samples treated with *D. metel* extract had lower WL when compared to samples treated with *Euphorbia hirta*. This implied that *D. metel*-treated samples were more resistant, this corroborate the statement earlier made by the researcher that *D. metel* based bio-preservatives will do better than *E. hirta*based bio-preservatives. Hence, they were less susceptible to white rot infestation.

ANOVA for samples subjected to white rot infestation (Table 4.11) revealed that effect of preservative concentration levels and interactions (except Extracts and EXT * Conc.) were significant on WL. Furthermore, result on effect of concentrations on weight loss (Tables 4.13 and 114) revealed that WL of control was not the same as any of the concentration levels. Hence, the percentage WL of control (0.76) was different from all the treated samples. This implied that treated sample had resistance properties which were not the same with control. This finding is in line with the report given by Kaur *et al*. (2016) on how plant extracts worked as a preservative for bamboo samples.

Effect of sampling height on weight loss was shown in Tables 4.12 and 15 this report shows that weight loss of top was significantly different from other sampling heights. This implied that, on exposure of samples to white rot fungi, samples obtained from top portion of the bamboo culm resisted white rot attack in a significantly different way compared with

Table 4.11: The Recorded Result on Weight loss of bamboo samples infested by white rot fungus (g)

Extracts	**Sampling**	**Concentration**					**Pooled**
	height	25%	50%	75%	100%	C	**mean**
DM	Base	0.22 ± 0.11^{a}	0.20 ± 0.09^{a}	0.18 ± 0.12^{c}	0.45 ± 0.19^{a}	1.57 ± 0.09^{c}	0.52 ± 0.55
	Middle 1	0.27 ± 0.30^{ab}	0.22 ± 0.07^{ab}	0.19 ± 0.08^{a}	0.32 ± 0.20^{ab}	0.56 ± 0.23^{ab}	0.31 ± 0.23
	Middle 2	0.17 ± 0.05^{c}	0.29 ± 0.19^{c}	0.12 ± 0.01^{b}	0.29 ± 0.40^{c}	0.65 ± 0.45^{b}	0.30 ± 0.32
	Top	0.22±0.07a	0.16 ± 0.05^{d}	0.14 ± 0.03^{ab}	0.26 ± 0.08^{c}	0.45 ± 0.08^{a}	0.25 ± 0.13
	Pooled mean	**0.22±0.16**	**0.22±0.11**	**0.16±0.08**	**0.33±0.24**	**0.81±0.51**	**0.35±0.36**
EH	Base	0.22 ± 0.13^{a}	0.34 ± 0.15^{a}	0.22 ± 0.09^{a}	0.45 ± 0.19^{a}	0.63 ± 0.17^{ab}	0.35 ± 0.21
	Middle 1	0.34 ± 0.13^{ab}	0.17 ± 0.11^{b}	0.39 ± 0.25^{b}	0.32 ± 0.20^{ab}	0.66 ± 0.22^{b}	0.41 ± 0.27
	Middle 2	0.65 ± 0.64^{c}	0.41 ± 0.07^{a}	0.35 ± 0.40^{b}	0.29 ± 0.40^{c}	1.00 ± 0.53^{c}	0.56 ± 0.46
	Top	0.14 ± 0.18^{c}	0.25 ± 0.13^{c}	0.17 ± 0.11^{c}	0.26 ± 0.08^{c}	0.54 ± 0.10^{a}	0.30 ± 0.20
	Pooled mean	**0.34±0.38**	**0.29±0.15**	**0.28±0.24**	**0.33±0.24**	**0.71±0.33**	**0.40±0.31**

Note: mean values with the same alphabets on the same column are not significantly different ($p\leq0.05$)

Table 4.12: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Sampling Height (SH) and Extract types of bamboo after exposure to White Rot

S/N	SH	Dm (g)	Eh (g)
1	Base	0.52 ± 0.55^{a}	0.35 ± 0.21^{bc}
2	Middle 1	0.31 ± 0.23^{c}	0.41 ± 0.27^{b}
3	Middle 2	0.30 ± 0.32^{c}	0.56 ± 0.46^{a}
4	Top	0.25 ± 0.13^{d}	0.30 ± 0.20^{c}

Note: mean values with the same alphabets are not significantly different ($p\leq0.05$)

Table 4.13: Effect of preservative concentration on weight loss (Pooled mean) on the basis of Conc. Level % and Extract types of bamboo after exposure to White Rot

S/N	Conc. Level	Dm (g)	Eh (g)
1	25	0.22 ± 0.16^{b}	0.34 ± 0.38^{c}
2	50	0.22 ± 0.11^{b}	0.29 ± 0.15^{bc}
3	75	0.16 ± 0.08^{a}	0.28 ± 0.24^{bc}
4	100	0.33 ± 0.24^{c}	0.33 ± 0.24^{c}
5	Control	0.81 ± 0.51^{d}	0.71 ± 0.33^{d}

Note: mean values with the same alphabets are not significantly different ($p\leq0.05$)

Table 4.14: Effect of concentration on weight loss

Concentration	Mean values
25%	0.28^{ab}
50%	0.25^{ab}
75%	0.22^{a}
100%	0.36^{b}
Control	0.76^{c}

Note: same letters refers to not significantly different

Table 4.15: Effect of sampling height on weight loss

Sampling height	Mean values
Top	0.27^{c}
Middle 1	0.36^{ab}
Middle 2	0.43^{b}
Base	0.43^{b}

Note: same letters refers to not significantly different

middle1, middle 2 and base. This could be attributed to variation in lignocellulosic composition such as vascular bundles and parenchyma of *B. vulgaris* along axial direction (Shukla, *et al.*, 1988).

4.5 Effect of bio-preservative on bamboo laminated board

4.5.1 Shear strength test for laminated bamboo samples treated with bio-preservatives

Table 4.16 show the result of the strength on the effect of bio-preservative treated laminated bamboo samples. The shear strength of the treated bamboo laminated (BLB) board samples ranged from 3.85 N/mm (75% preservative concentration) to 6.06 N/mm (25% preservative concentration) and 4.44 N/mm (25%, 50% and 100% preservative concentration) to 5.60 N/mm (Control) for *D. metel*-and *E. hirta*-treated samples respectively.

Results revealed that for *D. metel*-treated samples, highest shear strength (6.06 N/mm) was recorded for samples at concentration 25% while lowest shear strength value (3.85 N/mm) was recorded at concentration 75%. More so, shear strength of Control was 6.05 N/mm (next to samples treated at 25%). It was observed that resistance of samples to load (shear stress) along glue line for samples treated at higher concentrations were recognised to be lower than the untreated samples. This implied that delamination of elements was higher with laminates treated at 50%, 75% and 100% when compared with control. This could be due to interference on glue line of laminates at higher preservative concentration leading to reduction in bonding strength or adhesive potentials of the adhesive. This was contrary to the submission of Gaspar *et al.,* (2009) that shear strength of the glue lines of preservative treated wood was slightly higher than for untreated wood.

For *E. hirta*-treated samples Control had highest (5.13 N/mm) shear strength on glue line. This was followed by samples treated with preservative at 75%. Samples treated at 25%, 50% and 100% had shear strength of 4.44 N/mm each. Similar to samples treated with *D. metel*, treatment with preservative caused reduction of adhesive forces between the glue lines. Hence, control had highest shear strength. Although, samples treated with preservatives at 75% had high shear strength, highest shear strength was recorded for untreated samples. The bar chart (Figure 18) illustrates that higher shear strength were observed when laminated samples were not treated or when samples were treated at low preservative concentration.

The ANOVA for shear strength of samples revealed that effect of preservatives was not significant on shear strength. More so, preservative concentration had no significant effect on the result (Table 4.17).

Table 4.16: Shear strength (N/mm) of the two different preservatives

S/N	Concentration	*D. metel*	*E. hirta*
1	25%	6.06[a]	4.44[c]
2	50%	4.56[b]	4.44[c]
3	75%	3.85[c]	5.13[ab]
4	100%	4.80[b]	4.44[c]
5	Control	6.05[a]	5.60[a]

Note: same letters refers to not significantly different

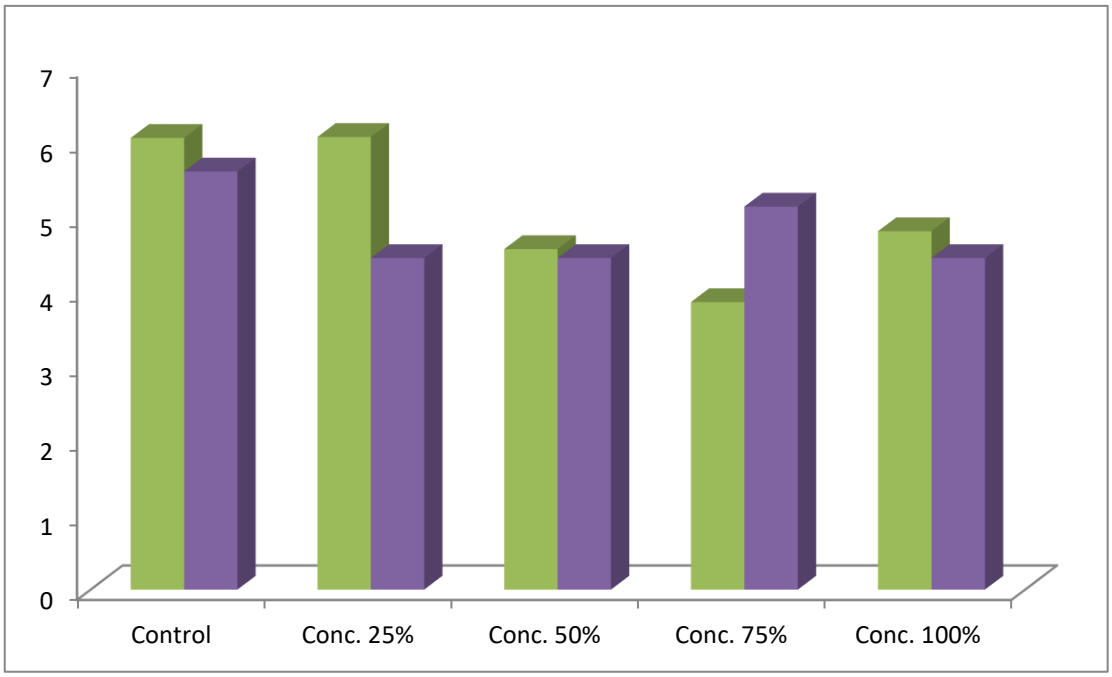

Figure 4.1: Graphical representation of the combined two mean results for comparison

Table 4.17: ANOVA for preservation at varying concentration

Source of Var.	df	SS	MSS	F	Sig.
Presv.	1	0.900	0.900	0.405	0.529ns
Presv. Conc.	4	7.478	1.870	0.842	0.509ns
Interaction	4	8.991	2.248	1.014	0.416ns
Error	30	66.531	2.218		
Corrected Total	39	83.900			

Ns means not significant

4.6 Fixation Experiment

The result of the leach-ability experiment shows that the chemical compounds present, Table 4.16 in the formulated preservatives has exhibited ability to deactivate the activities of degradable agents, however, it cannot fix in the vascular bundle of the bamboo. This corroborate the submission by Kaur *et al* (2016) made that bamboo treated with eco-friendly preservative will only last for about six months if place of service is out door.

CHAPTER FIVE

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.1 Summary

Lignocellulosic materials are versatile in pulp and paper, construction and so many other industries, however, to avert the challenges associated with bio-degradation it therefore require preservation to enhance durability for various applications. The toxic wtrf0nature of synthetic preservatives is encouraging the use of bio-preservative. Bio-preservatives from lignocellulosic extracts though a viable alternative but are not readily available nor their efficacy fully known. This study was therefore designed to evaluate the efficacy of extracts of *Datura metel (Dm)* and *Euphorbia hirta (Eh)* as bio-preservatives for *Bambusa vulgaris (Bv)* against termite and fungi attacks as well as its effect on the laminated board strength. Consequently, this study requires the following activities; the experimental samples of *Datura metel*, *Ephorbia hirta* and *Bambusa vulgaris* were sourced in Ibadan, Nigeria, and the plants (3) were authenticated in Herbarium of the Forestry Research Institute of Nigeria. The generated extracts from *Dm* and *Eh* were screened using reagents (phytochemical screening) to know the metabolite makeup and characterisation of the extracts were determined by utilising gas chromatography and mass spectrometry, to determine chemical constituents using AOAC (2000) procedures.

Bio-preservatives were formulated from extracts at 0, 25, 50, 75 and 100% concentrations using ASTM procedures. The 600 bamboo experimental samples were generated from top, middle 1, middle 2 and base of the five harvested *Bv* culms. The *Bv*

sample strips are of dimension (60×20×4 mm) and they were soaked in the 8 different concentrated preservatives (4 from each extract) for 72 hours, but were weighted before (initial weight) treatment in the bio-preservatives. Thereafter, the treated samples were conditioned and weight again (final weight). Treated samples and control were sub-divided into A, B and C groups (200 each). Group A was subjected to termite *(Macrotermis bellicosus)* attacks for 12 months, after which the exhume samples were conditioned and weighted, while groups B and C were subjected to brown *(Sclerotium rolfsii)* and white rot *(Pleurotus florida)* fungi for 6 months in accordance with ASTM procedures respectively, and sample were conditioned and weighted. Weight Loss (WL) analyses were done to evaluate the potency of the formulated bio-preservatives, while its effect on gluability of Bamboo Laminated Block (BLB) were also investigated using ASTM test methods. The gathered results were processed with the use of descriptive statistics and ANOVA at $\alpha_{0.05}$.

5.2 Conclusions

From the search done, the following conclusions were established

i. Additional baseline information regarding the efficacy of bio-preservatives (natural preservatives derived from plant's leaves, seeds, fruits wastes and essential oils) in preserving lignocellulosic materials like woody grass (bamboo).The study revealed that *Bambusa vulgaris* can be treated with bio-preservative generated from extracts from *Datura metel* and *Euphorbia hirta* in improving its resistance to degradation caused by bio-deteriorating agents such as insects and fungi.

ii. The extract yield weight of the two plants (*Datura metel* and *Euphorbia hirta*) using absolute ethanol as solvent was 1.85 g and 1.00 g, respectively. It was observed that, out of the two plants, *D. metel* had higher yield in terms of yield weight from extracts generated via absolute ethanol.

iii. According to Vanneste *et al.,*2002 and Maoz *et al.,* 2007, extracts derived from plants' leaves, seeds, fruit wastes and essential oils are explored as preservatives to protect lignocellulosic materials against agents of

biodegradation such as fungi and insects. The presence of some metabolites especially saponins, flavonoids and tannins in *D. metel* and *E.hirta* formed the bases on which the plants have preservative potential as reports by Pelczar *et al*; 1988.

iv. Gas chromatography screening of extract from *Datura metel,* enlisted the following chemical compounds; Dodecanoic acid, ethyl ester, Tetra decanoic acid, ethyl ester, Cyclohexanol, 2-(methylaminomethyl)-trans, Bicyclo [3.1.1] heplane, 2,6,6,-trimethyl, 2-Pentadecanone, 6,10,14- trimethyl, 2-6, 17-Octadecadien-1-01 acetate, 1,2 – Dihexylcyclo propene, 3,3 – Dimethyl - 4- methyl amino – butan-2- one. Similarly, *Euphorbia hirta*, contains such chemical compounds as, Bicycle (3.1.1) heptune, 2,6,6 –trimethyl (1α,2β,5α), 1-Alanine, N-(1-oxopentyl), methyl ester, 3-Azabicyclo (3-2-2) nonane, 11-Tetradecyn-1-01 acetate. Some of the chemical compounds have fungicidal properties with a high potential for bioaccumulation. They also serve as antifungal agrochemical. Therefore, based on the fact that there are more of flavonoids, tannins and other chemical compounds in *D. metel* than *E. hirta,* it is likely that bio-preservatives formulated from *D. metel* may resist activities of bio-deteriorating agents more than preservatives formulated from *E. hirta*.

v. Based on weight loss (%) evaluation between *D. metel and E. hirta*-treated samples, and statistical analysis conducted on collected data, shows that, there was large difference in efficacy of the formulated bio-preservatives for bamboo treatment against termite and fungi. Additionally, it was noted that there was a big difference in WL between preservative concentrations, but effect of sampling height was not significant on WL at $p \leq 0.05$. This implied that sampling heights at which samples were obtained before treatment with bio-preservative had no significant effect on WL of bamboo. Also, results revealed that, only WL of Control was different. Weight loss of treated samples at all concentration levels was the same. The implication was that,

the extent of termite attack on control was significantly different when compared to treat samples at all levels of concentrations.

vi. The follow-up test on ANOVA for Weight loss of bamboo experimental samples infested with brown rot fungus showa that all treatment effects and their interactions (except Conc. * SH and EXT* Conc * SH) were significant on WL. Furthermore, it was revealed that WL of control was different while that of treated samples at all concentration levels were the same. This implied that, percentage WL of control (untreated samples) was not the same as that of treated samples at all the concentration levels. Also, effect of sampling height on weight loss revealed that WL of base and Middle 1 were not the same with that of Middle 2 and top. The result implied that, after exposure to brown rot infestation, WL of samples obtained from base and middle 1 were not the same as that obtained at middle 2 and top. This could be attributed to variation in lignocellulosic composition such as vascular bundles and parenchyma of *B. vulgaris* along axial direction (Shukla, *et al.*, 1988).

vii. The follow up test on ANOVA for samples subjected to white rot infestation revealed that effect of preservative concentration levels and interactions (except Extracts and EXT * Conc.) were significant on WL. Furthermore, results on effect of concentrations on weight loss revealed that WL of Control was not the same as any of the concentration levels. Hence, the percentage WL of Control (0.76) was different from all the treated samples. This implied that treated sample had resistance properties which were not the same with control. This result is in line with Kaur *et al.* (2016) who describe on effectiveness of plant extracts as preservative for lignocellulosic materials. Also, effect of sampling height on weight loss revealed that Top was different from other sampling heights. This implied that, after exposure to White rot fungi, WL of samples obtained from top was not the same with middle1, middle 2 and base. This could be attributed to differential resistance along the axial axis caused by variation in lignocellulosic composition such as vascular bundles and parenchyma of *B. vulgaris* along axial direction.

viii. The follow-up test on ANOVA for shear strength of bamboo laminated board samples revealed that effect of natural preservatives was not significant on shear strength. More so, preservative concentration had no significant effect on the result.

ix. Results on leach-ability of natural preservatives revealed that the chemical compounds present in the formulated bio-preservatives has exhibited ability to deactivate the activities of bio-degradable agents and can successfully be chemically fixed to the woody grass.

5.3 Recommendations

Based on the result it is recommended that:

i. Further studies should be conducted as regards searching for other plants whose extracts has potentials for treating lignocellulosic materials. This will provide additional information for documentation and research frontier.

ii. Extracts should be generated from the two plants using N-Hexane, Toluene, and Distil water (solvents). The generated extracts should be screened through phytochemical screening and gas chromatography analysis to know the useful active bio-metabolites and chemical compounds presents for information and documentation.

iii. Since it was reported, that time of harvesting *Bambusa vulgaris* is a major factor influencing durability of bamboo culms in storage and service; it is recommended that natural durability test should be carried out on both dry and raining season harvested bamboo to confirm that claim.

iv. This study has provided information that at 75% concentration of the selected plants extracts (*Datura metel* and *Euphorbia hirta*) performed better in the treatment of *Bambusa vulgaris* against termite and fungi. It is recommended that more study be done to know the exact quantitative phytochemicals of the metabolites that actually performed the best, and so that the chemical compounds can be synthesized for mass production.

v. It was reported that *Datura metel* formulated bio-preservative at concentration 75% deactivated the activities of white rot fungus while *Euphorbia hirta* formulated bio-preservative at the same level of concentration deactivate activities of brown rot fungus. Further study be carried out on the combination of the two bio-preservative to evaluate its potency on the two fungi (it will end up been a bio-preservative deactivating two different lignocellulosic material destroying fungi).

vi. Again, more research is required on how the quantum of metabolites and the active chemical compounds be retained (to avoid leaching) in lignocellulosic materials to achieve longer service life.

vii. Considering the result of the shear strength on glue line of treated bamboo laminated board (BLB), in order to further expand the scope of study, effect of heat or pressure on glue line before it is exposed to load for shear strength test can be investigated through varying the curing temperature or clamping pressure.

viii. Further studies should be conducted as regards determining other plants whose extracts have potentials for treating lignocellulosic materials. This will provide additional information for documentation and research frontier.

ix. Extracts should be generated from the two plants using N-Hexane, Toluene, and Distil water. The generated extracts should be screened through phytochemical screening and gas chromatography analysis to know the useful active bio-metabolites and chemical compounds presents for information and documentation.

x. Since it was reported, that time of harvesting *Bambusa vulgaris* is a major factor influencing durability of bamboo culms in storage and service; it is recommended that natural durability test should be carried out on both dry and raining season harvested bamboo to confirm that claim.

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xiii. Again, more research is required on how the quantum of metabolites and the active chemical compounds be retained (to avoid leaching) in lignocellulosic materials to achieve longer service life.

xiv. Considering the result of the shear strength on glue line of treated bamboo laminated board (LBL), in order to further expand the scope of study, effect of heat or pressure on glue line before it is exposed to load for shear strength test can be investigated through varying the curing temperature or clamping pressure.

xv. Additional baseline information regarding the efficacy of bio-preservatives (natural preservatives derived from plant's leaves, seeds, fruits wastes and essential oils) in preserving lignocellulosic materials like woody grass (bamboo).The study revealed that *Bambusa vulgaris* can be treated with bio-preservative generated from extracts from *Datura metel* and *Euphorbia hirta* in improving its resistance to degradation caused by bio-deteriorating agents such as insects and fungi.

5.4 Contributions to knowledge

This study has made contribution to knowledge in the following fourteen areas:

i. The bio-preservatives formulated from *Datura metel* and *Euphorbia hirta* extracts offered adequate protection to bamboo against termite and fungi

attacks. They are suitable bio-preservatives for lignocellulosic materials, being a substitute to the inorganic-based preservatives.

ii. Extracts obtained using ethanol as solvent from *D. metel* and *E. hirta* plants at 75% concentration (following the preparatory method used in this research) can be used as bio-preservatives for *B. vulgaris* also for other lignocellulosic materials against bio-deteriorating agents such as termite (*Macrotermis bellicosus*), brown rot (*Sclerotium rolfsii*) and white rot (*Pleurotus florida*).

iii. The extract yield weight of the two plants (*Datura metel* and *Euphorbia hirta*) using absolute ethanol as solvent was 1.85 g and 1.00 g, respectively. It was observed that, out of the two plants, *D. metel* had higher yield in terms of yield weight from extracts generated via absolute ethanol.

iv. According to Vanneste *et al.*,2002 and Maoz *et al.*, 2007, extracts derived from plants' leaves, seeds, fruit wastes and essential oils are explored as preservatives to protect lignocellulosic materials against agents of biodegradation such as fungi and insects. The presence of some metabolites especially saponins, flavonoids and tannins in *D. metel* and *E.hirta* formed the bases on which the plants have preservative potential as reports by Pelczar *et al*; 1988.

v. It was reported that *Datura metel* formulated bio-preservative at concentration 75% deactivated the activities of white rot fungus while *Euphorbia hirta* formulated bio-preservative at the same level of concentration deactivate activities of brown rot fungus.

vi. Gas chromatography screening of extract from *Datura metel,* enlisted the following chemical compounds; Dodecanoic acid, ethyl ester, Tetra decanoic acid, ethyl ester, Cyclohexanol, 2-(methylaminomethyl)-trans, Bicyclo [3.1.1] heplane, 2,6,6,-trimethyl, 2-Pentadecanone, 6,10,14- trimethyl, 2-6, 17-Octadecadien-1-01 acetate, 1,2 – Dihexylcyclo propene, 3,3 – Dimethyl - 4- methyl amino – butan-2- one. Similarly, *Euphorbia hirta*, contains such chemical compounds as, Bicycle (3.1.1) heptune, 2,6,6 –trimethyl (1α,2β,5α),

1-Alanine, N-(1-oxopentyl), methyl ester, 3-Azabicyclo (3-2-2) nonane, 11-Tetradecyn-1-01 acetate. Some of the chemical compounds have fungicidal properties with a high potential for bioaccumulation. They also serve as antifungal agrochemical. Therefore, based on the fact that there are more of flavonoids, tannins and other chemical compounds in *D. metel* than *E. hirta*, it is likely that bio-preservatives formulated from *D. metel* may resist activities of bio-deteriorating agents more than preservatives formulated from *E. hirta*.

vii. Based on weight loss (%) evaluation between *D. metel and E. hirta*-treated samples, and statistical analysis conducted on collected data, shows that, there was large difference in efficacy of the formulated bio-preservatives for bamboo treatment against termite and fungi. Additionally, it was noted that there was a big difference in WL between preservative concentrations, but effect of sampling height was not significant on WL at $p \leq 0.05$. This implied that sampling heights at which samples were obtained before treatment with bio-preservative had no significant effect on WL of bamboo. Also, results revealed that, only WL of Control was different. Weight loss of treated samples at all concentration levels was the same. The implication was that, the extent of termite attack on control was significantly different when compared to treat samples at all levels of concentrations.

viii. The follow-up test on ANOVA for Weight loss of bamboo experimental samples infested with brown rot fungus shown that all treatment effects and their interactions (except Conc. * SH and EXT* Conc * SH) were significant on WL. Furthermore, it was revealed that WL of control was different while that of treated samples at all concentration levels were the same. This implied that, percentage WL of control (untreated samples) was not the same as that of treated samples at all the concentration levels. Also, effect of sampling height on weight loss revealed that WL of base and Middle 1 were not the same with that of Middle 2 and top. The result implied that, after exposure to brown rot infestation, WL of samples obtained from base and middle 1 were

not the same as that obtained at middle 2 and top. This could be attributed to variation in lignocellulosic composition such as vascular bundles and parenchyma of *B. vulgaris* along axial direction (Shukla, *et al.*, 1988).

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x. The follow-up test on ANOVA for shear strength of bamboo laminated board samples revealed that effect of natural preservatives was not significant on shear strength. More so, preservative concentration had no significant effect on the result.

xi. Results on leach-ability of natural preservatives revealed that the chemical compounds present in the formulated bio-preservatives has exhibited ability to deactivate the activities of bio-degradable agents but cannot be chemically fixed to the woody grass.

xii. For optimum utilisation of bamboo for construction purposes, the base, middle 1 and middle 2 maybe preferred after treating with bio-preservative at

the required concentration, while utilisation of the top portion of bamboo may be considered for application where risk of structural failure is low.

xiii. Strips from the base, middle 1, and middle 2 should be utilised in the manufacture of laminated bamboo boards (BLB) in order to produce a material that is less hygroscopic, durable and of superior strength.

xiv. The result on the shear strength on glue line of treated bamboo laminated board (BLB) shows that there was no significant different, therefore, no negative effect of glue adhesive properties as a result of bio-preservative treatment of bamboo stripes vis a vis laminating exercises during the production of bamboo laminated board (BLB).

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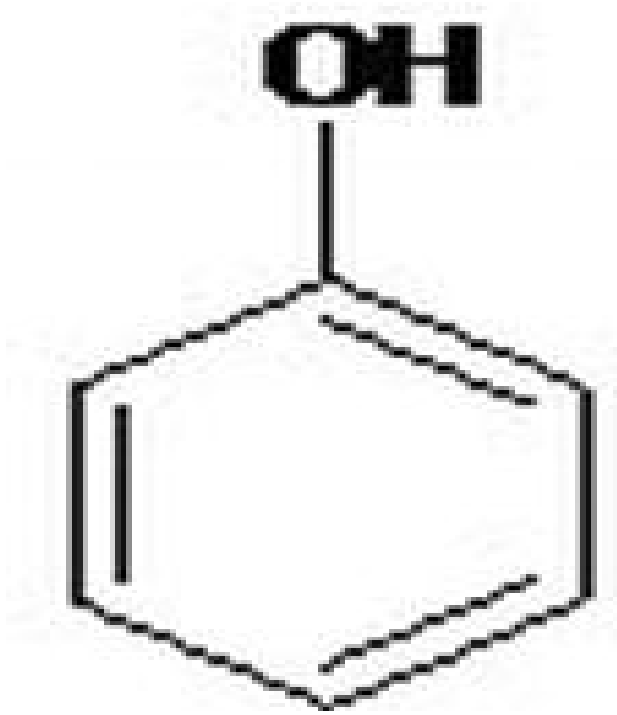
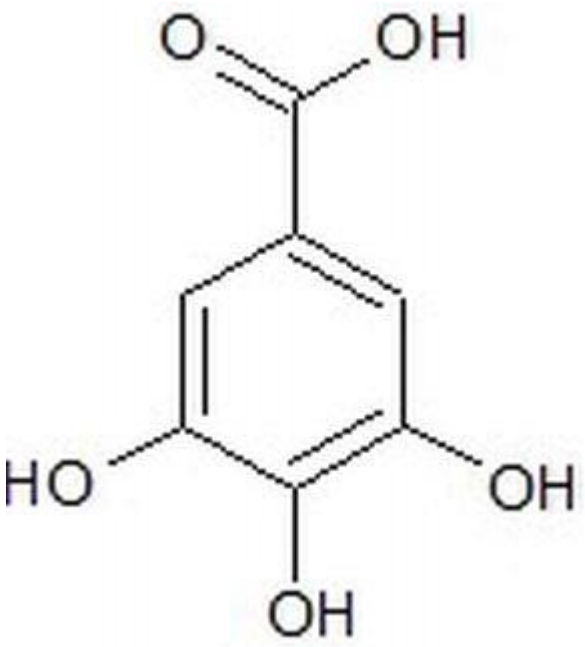
APPENDICES



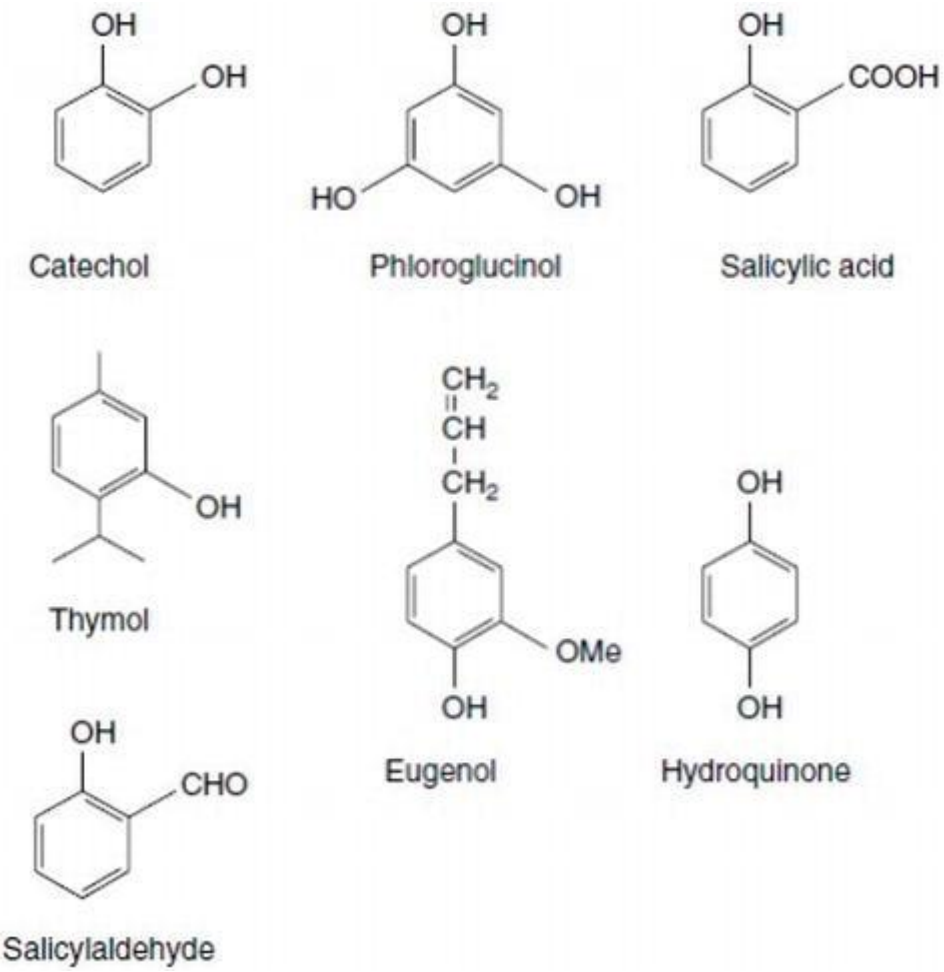
Appendix 1. Phenol. single-aromatic ring simple structures

Source;https://en.m.org



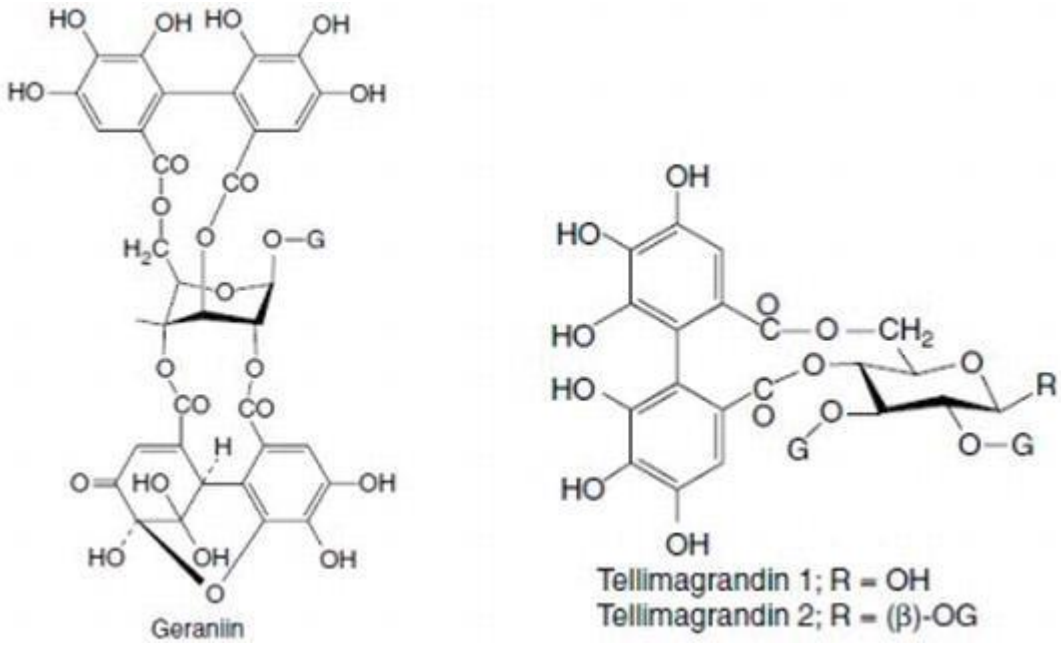
Appendix 2. For its astringent qualities, gallic acid is widely known

Source;https://en.m.org



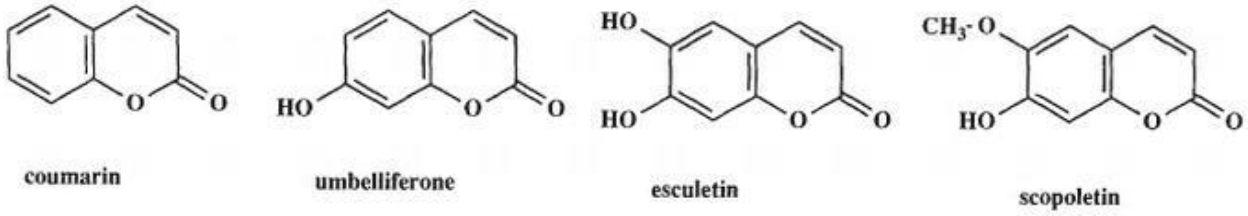
Appendix 3. Examples of simple phenolics.

Source; https://en.m. org



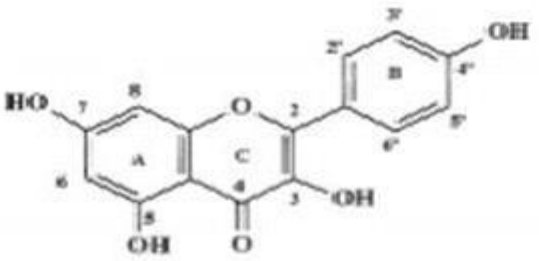
Appendix 4. Examples of tannins that are hydrolysable.

Source;https://en.m..org

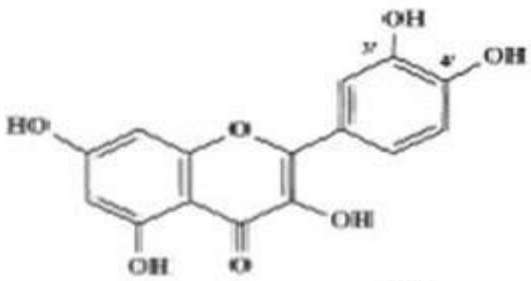


Appendix 5. Examples of coumarins.

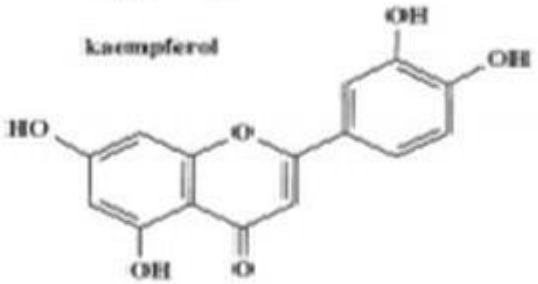
Source; https://en.m.org



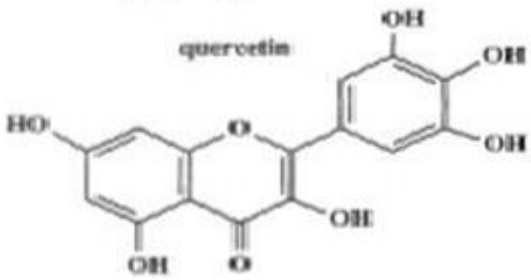
kaempferol



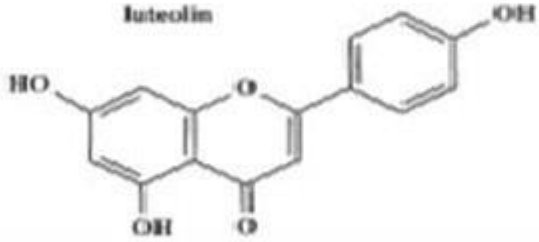
quercetin



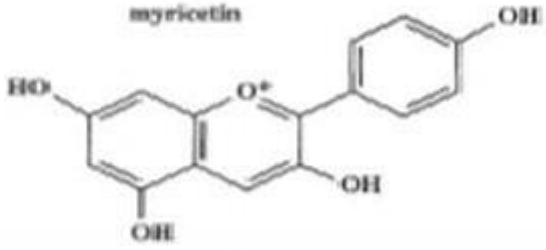
luteolin



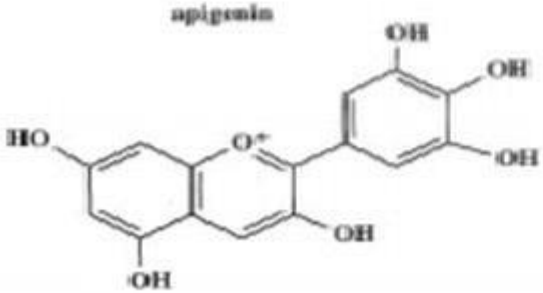
myricetin



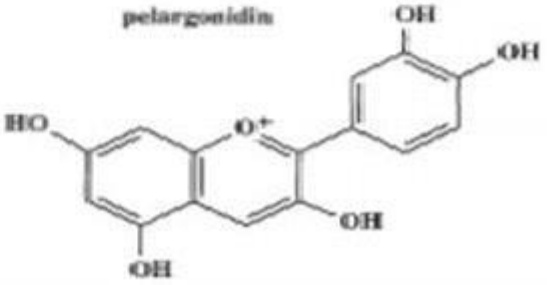
apigenin



pelargonidin



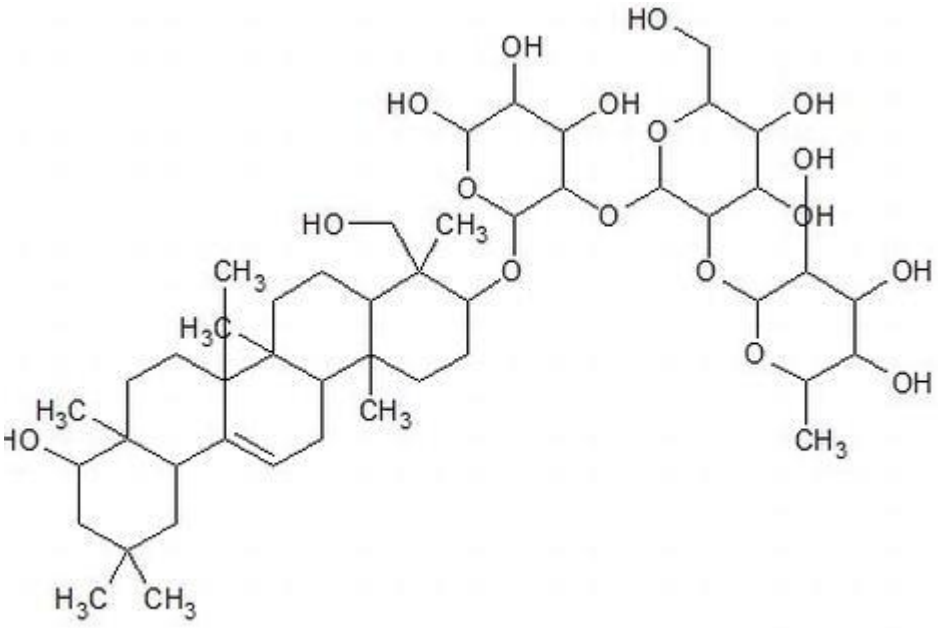
delphinidin



cyanidin

Appendix 6. Examples of flavonoids known for its ability to reduce inflammation and allergies.

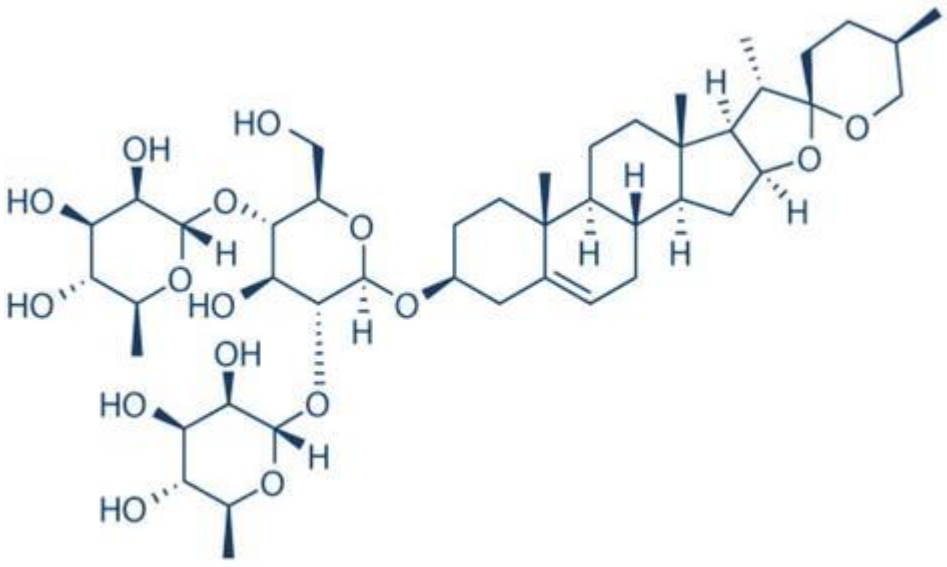
Source;https://en.m.org



Soyasaponin I

Appendix 8. An illustration of triterpenoidal saponin These sugar units can be made of pentoses, hexoses, or uronic acids.

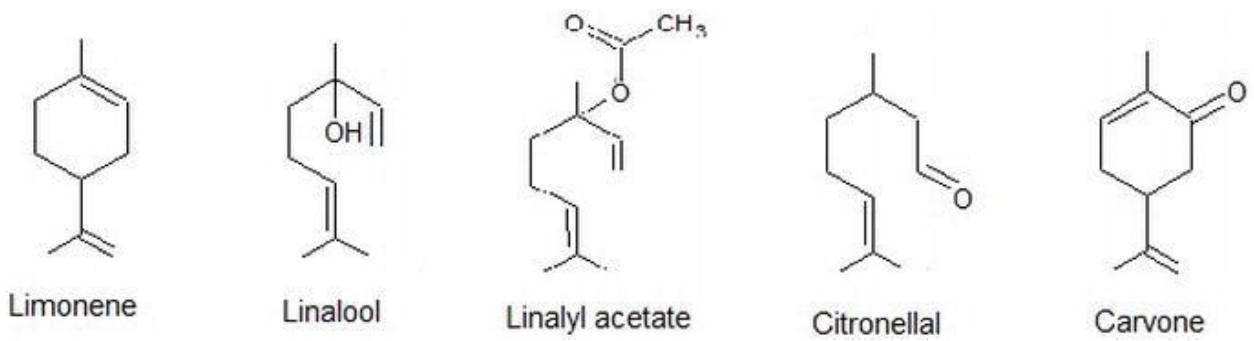
Source; https://en.m. org



Dioscin

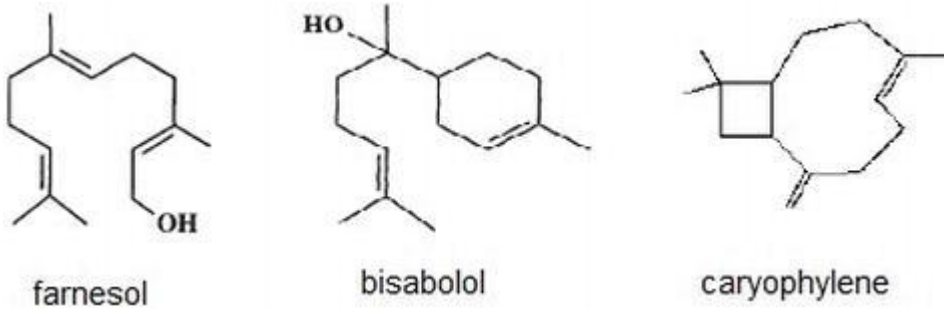
Appendix 9. Example of steroidal saponin, These sugar units can be made of uronic, pentose, or hexose acids.

Source;https://en.m. org



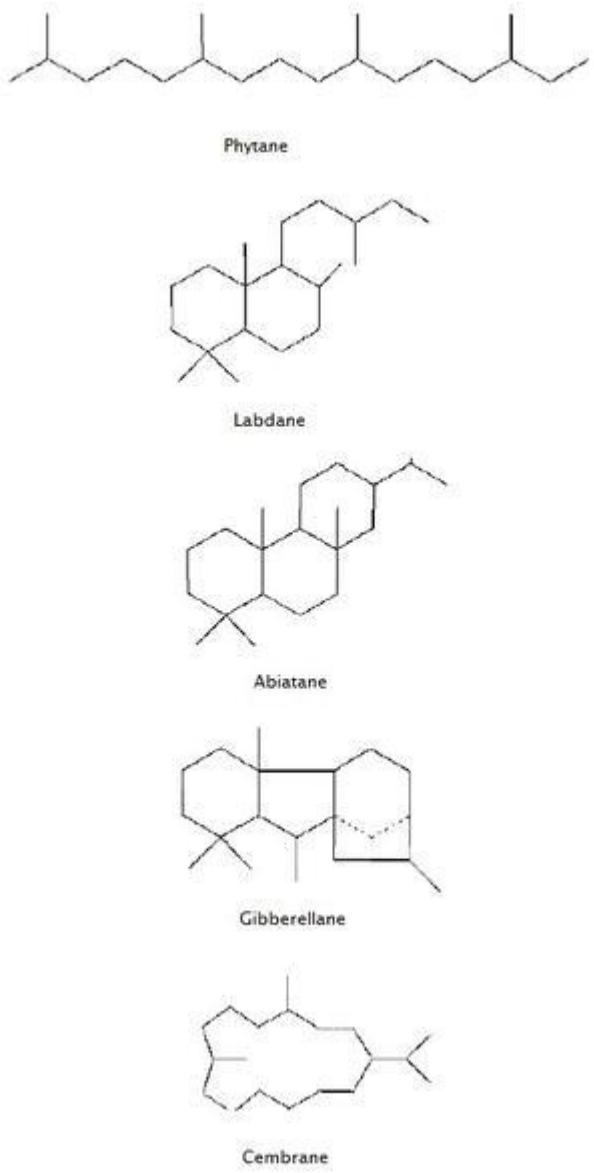
Appendix 10. Examples of monoterpenes, essential elements of volatile oils or essential oils from plants.

Source; https://en.m. org



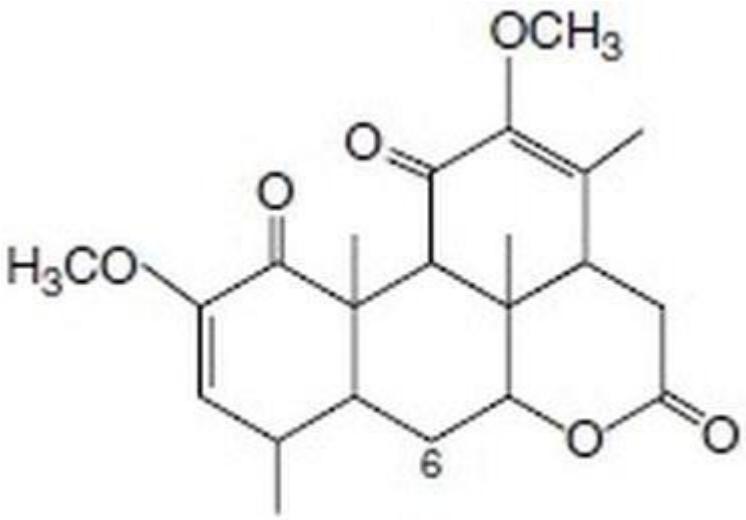
Appendix 11. Examples of sesquiterpenes, exhibit antibacterial, antifungal, and antiprotozoan properties.

Source;https://en.m. org



Appendix 12. Examples of diterpenes, It displayed a variety of pharmacological qualities, in addition to analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic, and antiprotozoal actions.

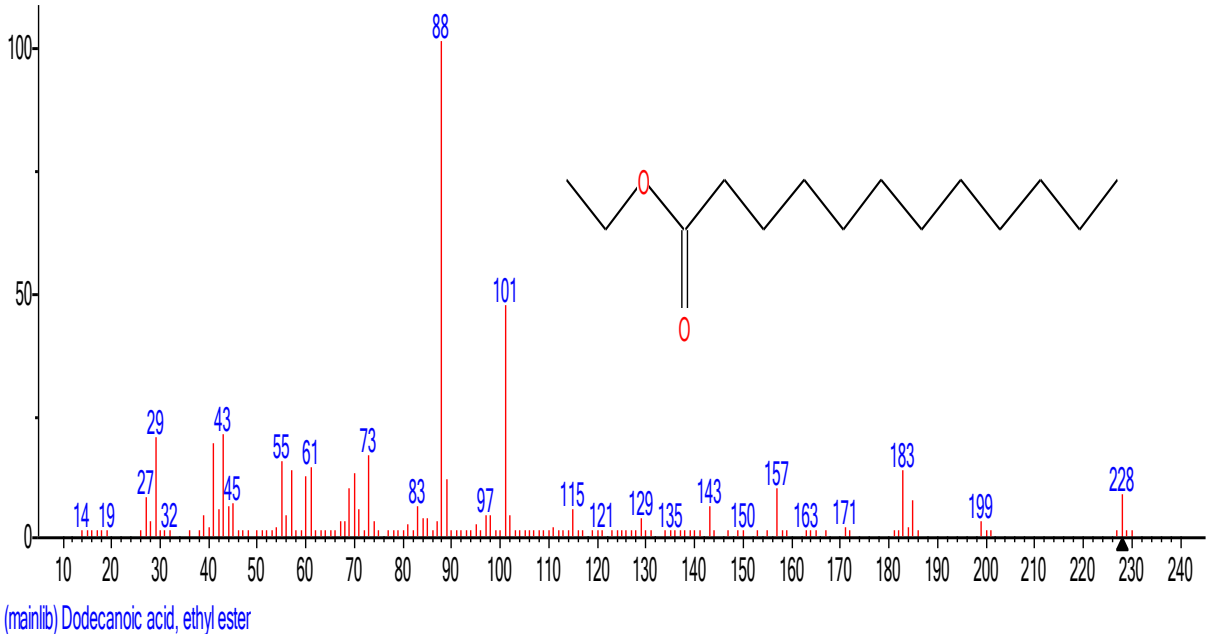
Source; https://en.m. org



Quassin

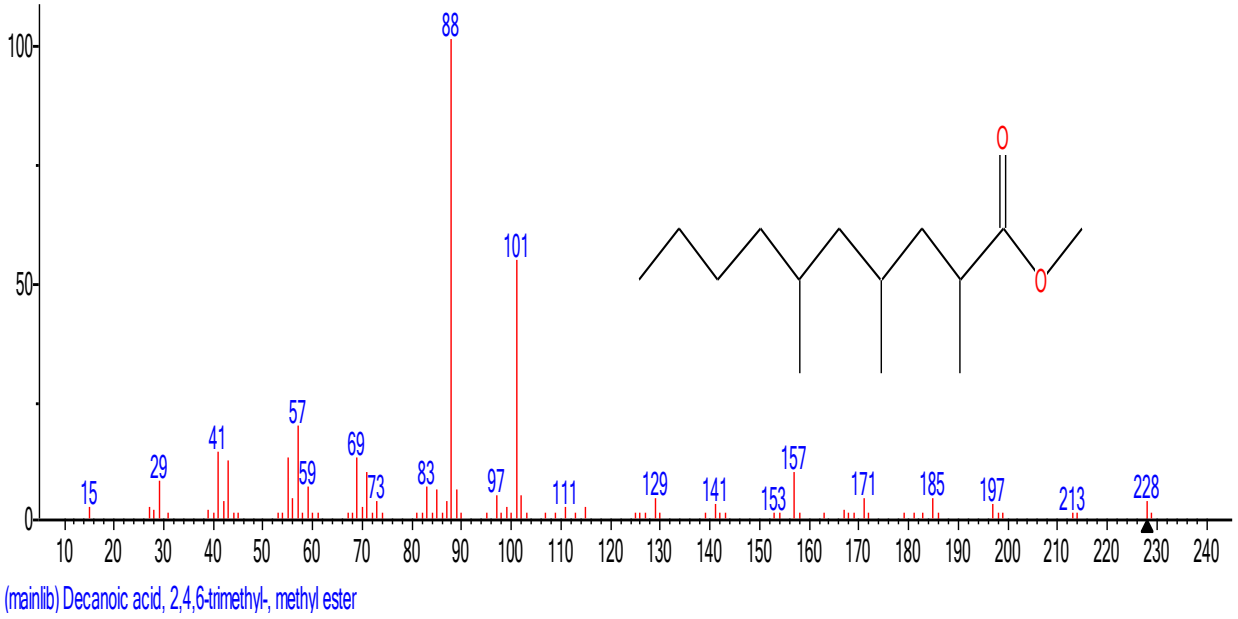
Appendix 13. Example of triterpene, These substances serve as both plant and animal steroid precursors.

Source; https://en.m. org



Appendix 14: Dodecanoic acid, ethyl ester

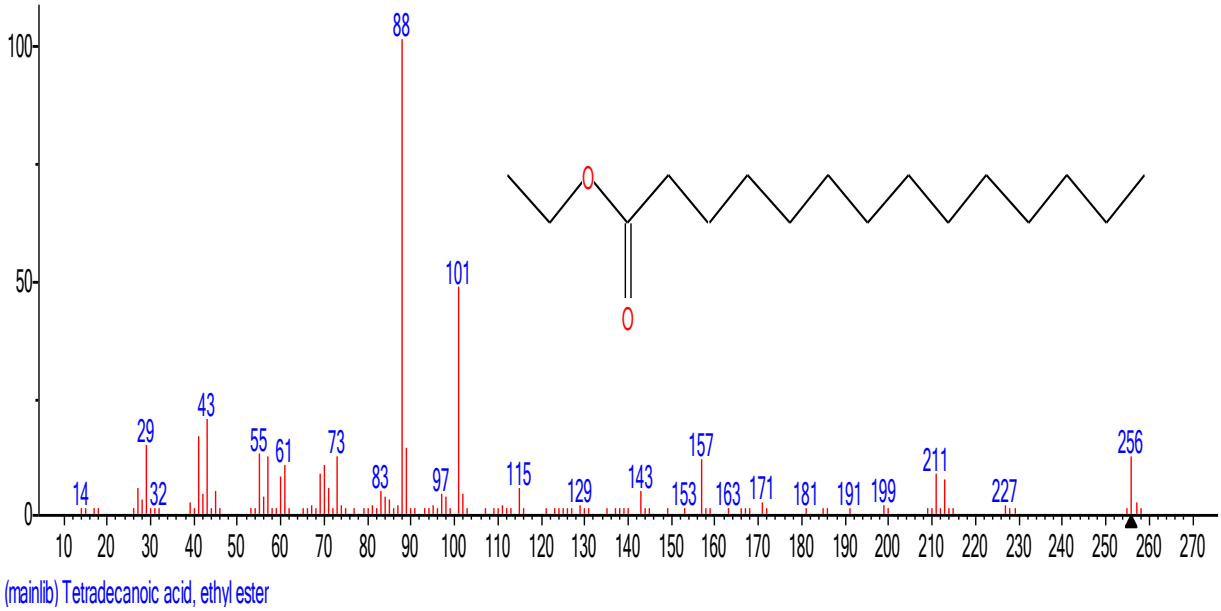
Dodecanoic acid, ethyl ester, IUPAC NAME = ethyl dodecanoate, Molecular Weight: 228.37 g/mol. Ethyl dodecanoate is found in alcoholic beverages. Ethyl dodecanoate is present in various fruits, e g. apple, apricot, guava, melon, etc. Also present in wheat bread, crisp bread, ginger, whisky, fruit brandies and wine flavouring agent.



Appendix 15: Decanoic acid, 2,4,6-trimethyl- methyl ester

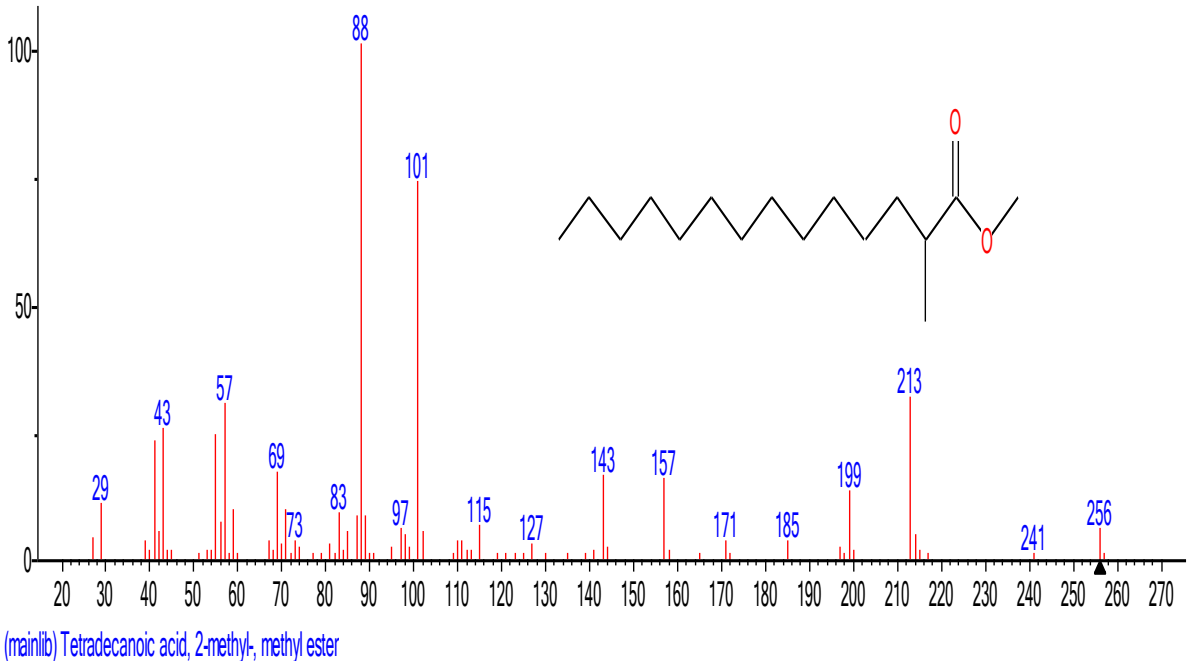
Ethyl laurate also known as decanoic acid ($C_{14}H_{28}O_2$), 2,4,6-trimethyl- methyl ester, IUPAC NAME = methyl 2,4,6-trimethyldecanoate, Molecular Weight: 228.37 g/mol

Found in alcohol beverages, in fruits (guava, aprocat, apple etc), in wine, it is a flavoring agent. It is not poisonous to human



Appendix 16: Tetradecanoic acid,ethyl ester

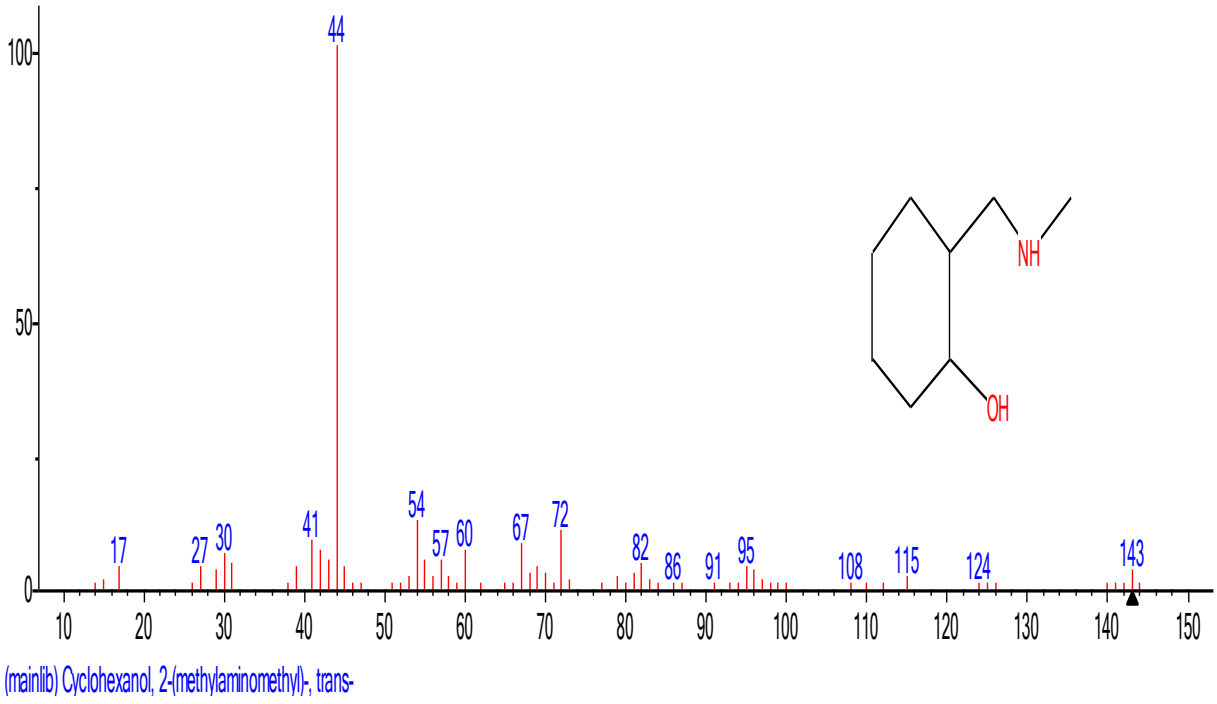
Tetradecanoic acid,ethyl ester, IUPAC NAME = ethyl tetradecanoate, Molecular Weight: 256.42 g/mol/ Ethyl tetradecanoate, also known as myristate ethyl ester or ethyl myristate, belongs to the class of organic compounds known as fatty acid esters. These are carboxylic ester derivatives of a fatty acid. Ethyl tetradecanoate is a very hydrophobic molecule, practically insoluble (in water), and relatively neutral, it is not poisonous to human



Appendix 17: Tetradecanoid acid, 2-methyl-, methyl ester

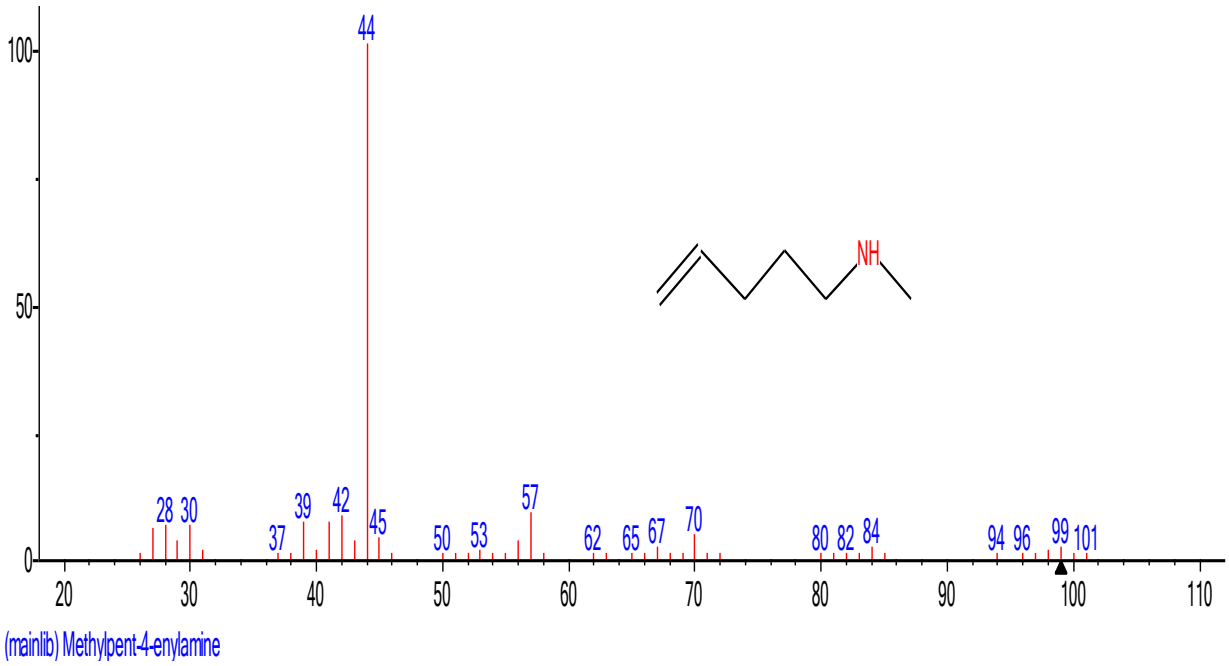
Tetradecanoic acid, 2-methyl-, methyl ester, IUPAC NAME = Methyl 2-methyltetradecanoate

Molecular Weight is 256.42 g/mol



Appendix 18: Cyclohexanol, 2-(methylaminoethyl)-, trans-

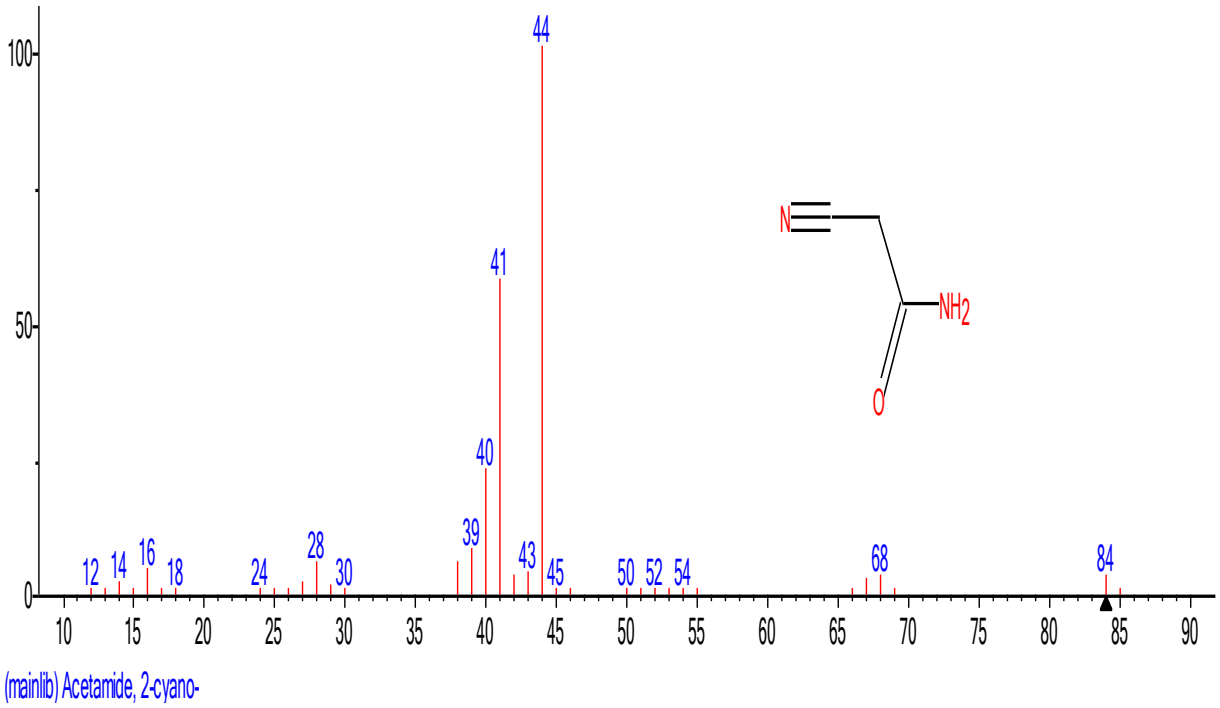
Cyclohexanol, 2-(methylaminoethyl)-, trans-, IUPAC NAME = 4-[2-(methylamino)ethyl] cyclohexan-1-ol, Molecular Weight: 157.25g/mol



Appendix 19: Methylpent-4-enylamine

Methylpent-4-enylamine, IUPAC NAME = *N*-methylpent-4-en-1-amine

Molecular Weight: 99.17 g/mol

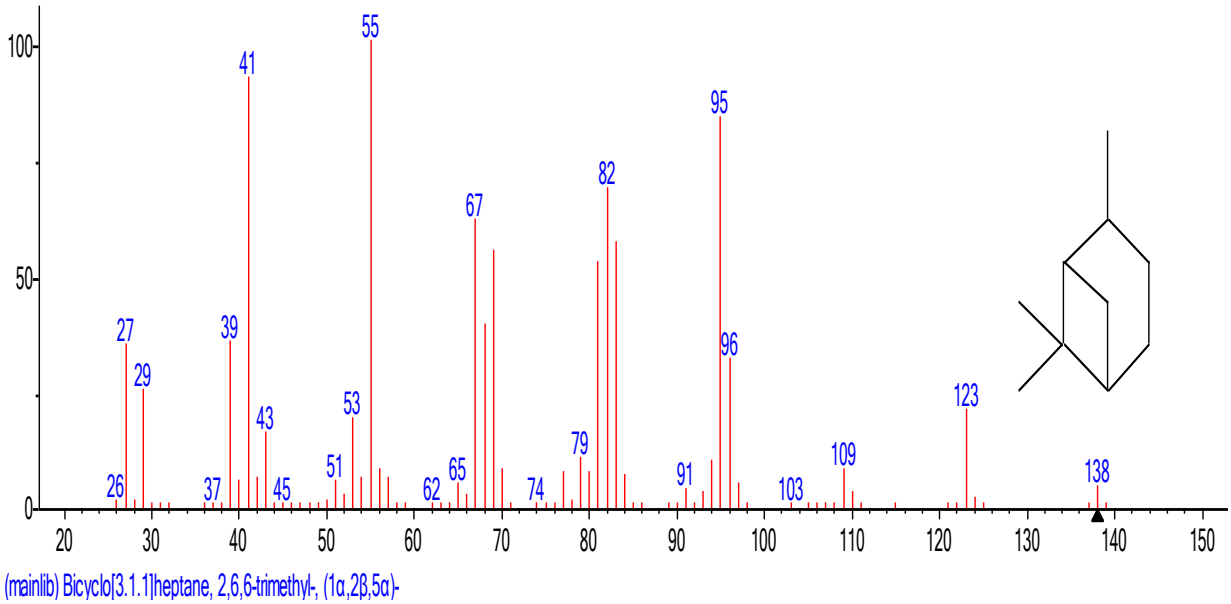


Appendix 20: Acetamide, 2-cyano

Activity: Acetamide, 2-cyano, IUPAC NAME = 2-cyanoacetamide, Molecular Weight: 84.08g/mol. Harmful if swallowed [Warning Acute toxicity, oral]H315 (100%): Causes skin irritation [Warning Skin corrosion/irritation]

H319 (100%): Causes serious eye irritation [Warning Serious eye damage/eye irritation]

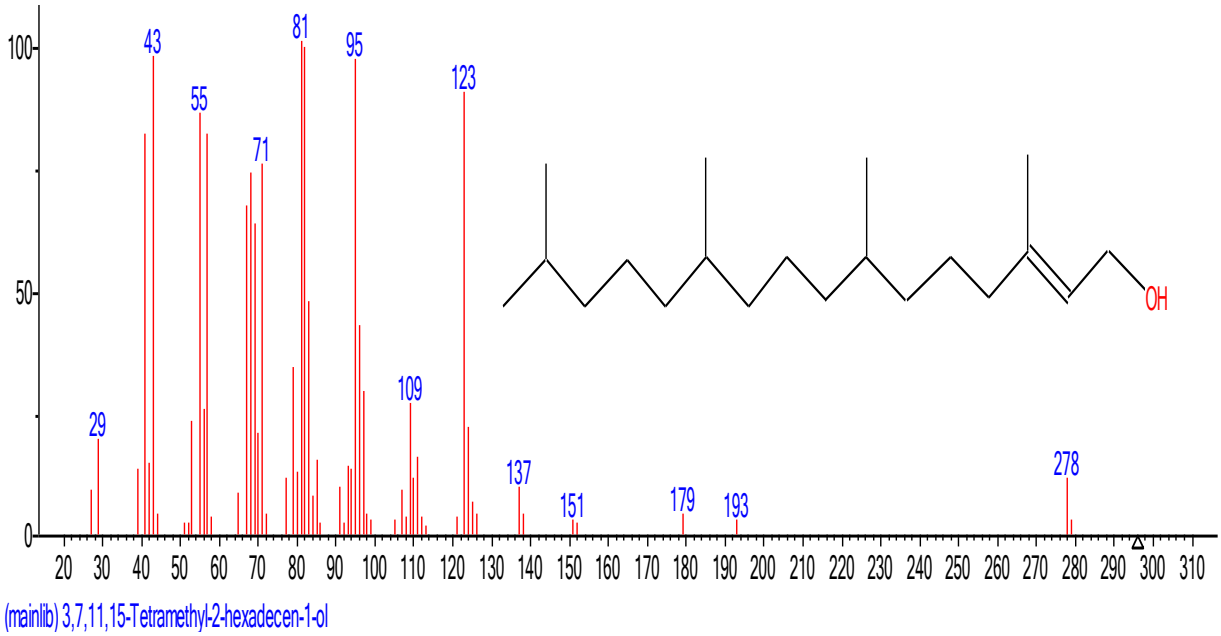
H335 (81.67%): May cause respiratory irritation [Warning Specific target organ toxicity, single exposure; Respiratory tract irritation]



Appendix 21: Bicycle(3.1.1) heptane, 2,6,6-trimethyl-, (1α,2β,5α)

Bicyclo(3.1.1) heptane, 2,6,6-trimethyl-, (1α,2β,5α), IUPAC NAME = (2,6,6-trimethyl-3-bicyclo[3.1.1]heptanyl) methanamine, Molecular Weight: 167.29 g/mol

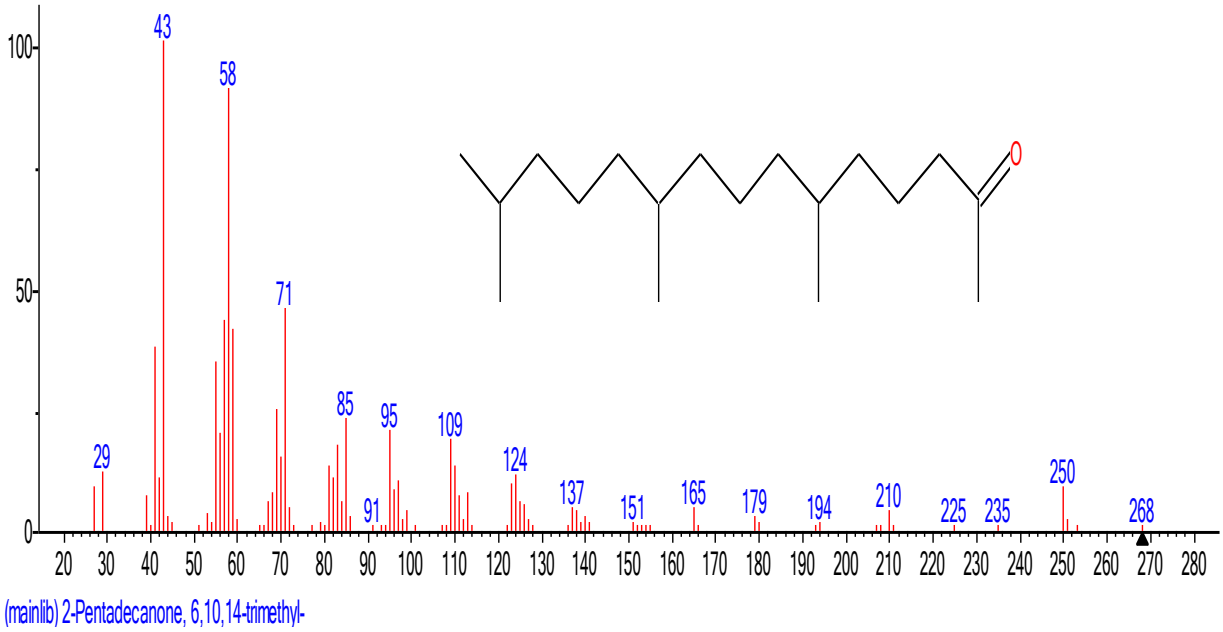
Activity: An active chemical under the Toxic Substances Control Act (TSCA) collections which contains information on chemicals and their regulation on ways to prevent and redu ce pollution, and on safer chemicals, products and practices.



Appendix 22: 3,7,11,15- tetramethyl-2-hexadecen-1-ol

3,7,11,15- tetramethyl-2-hexadecen-1-ol, IUPAC NAME = 3,7,11,15-tetramethylhexadecan-1-ol

Molecular Weight: 298.5 g/mol



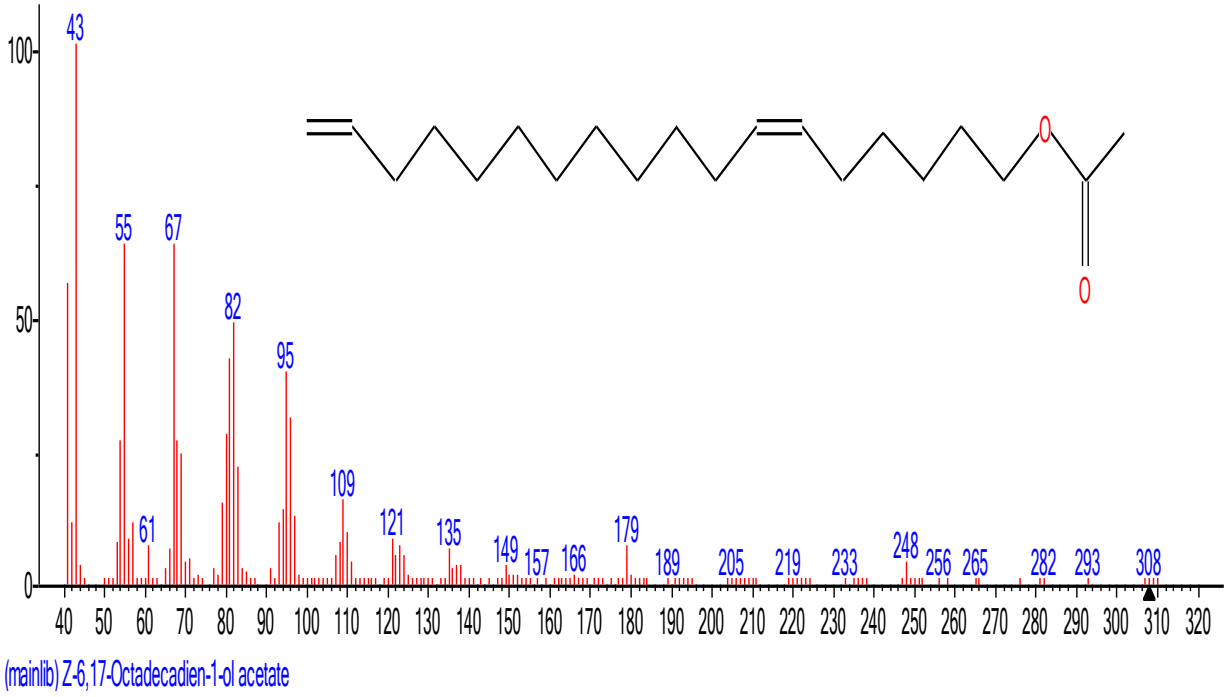
Appendix 23: 6,10,14-trimethyl-2-pentadecanone

6,10,14-trimethyl-2-pentadecanone, IUPAC NAME = 6,10,14-trimethylpentadecan-2-one

Molecular Weight: 268.5 g/mol, H400 (65.45%):

Activity: Very toxic to aquatic life [Warning Hazardous to the aquatic environment, acute hazard] H410 (65.45%): Very toxic to aquatic life with long lasting effects [Warning Hazardous to the aquatic environment, long-term hazard]

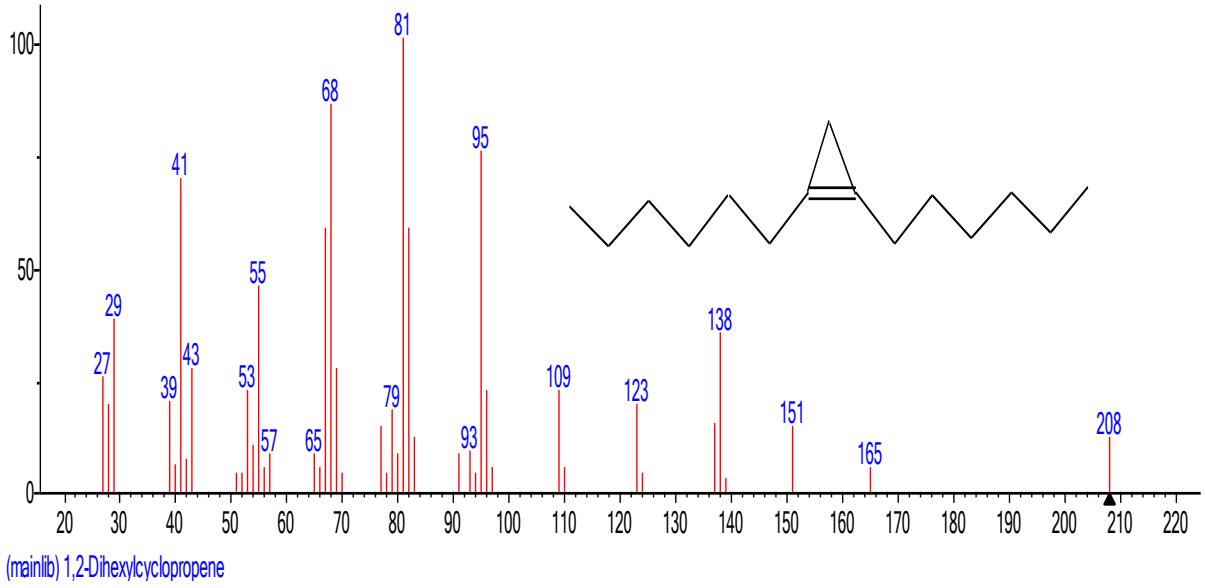
H413 (34.55%): May cause long lasting harmful effects to aquatic life [Hazardous to the aquatic environment, long-term hazard]



Appendix 24: Z-6,17-Octadecadien-1-ol acetate

Z-6,17-Octadecadien-1-ol acetate, IUPAC NAME =

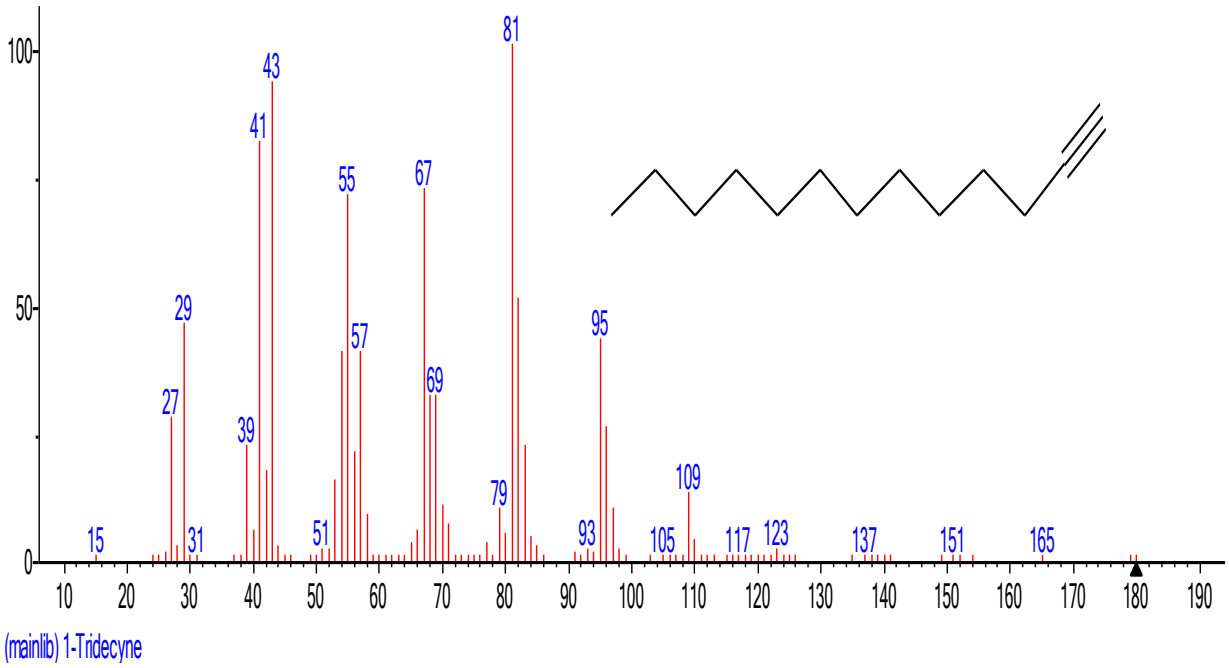
Molecular Weight



Appendix 25: 1,2-dihexcyclopropene

1,2-dihexcyclopropene, IUPAC NAME = 1,2-dihexylcyclopropene

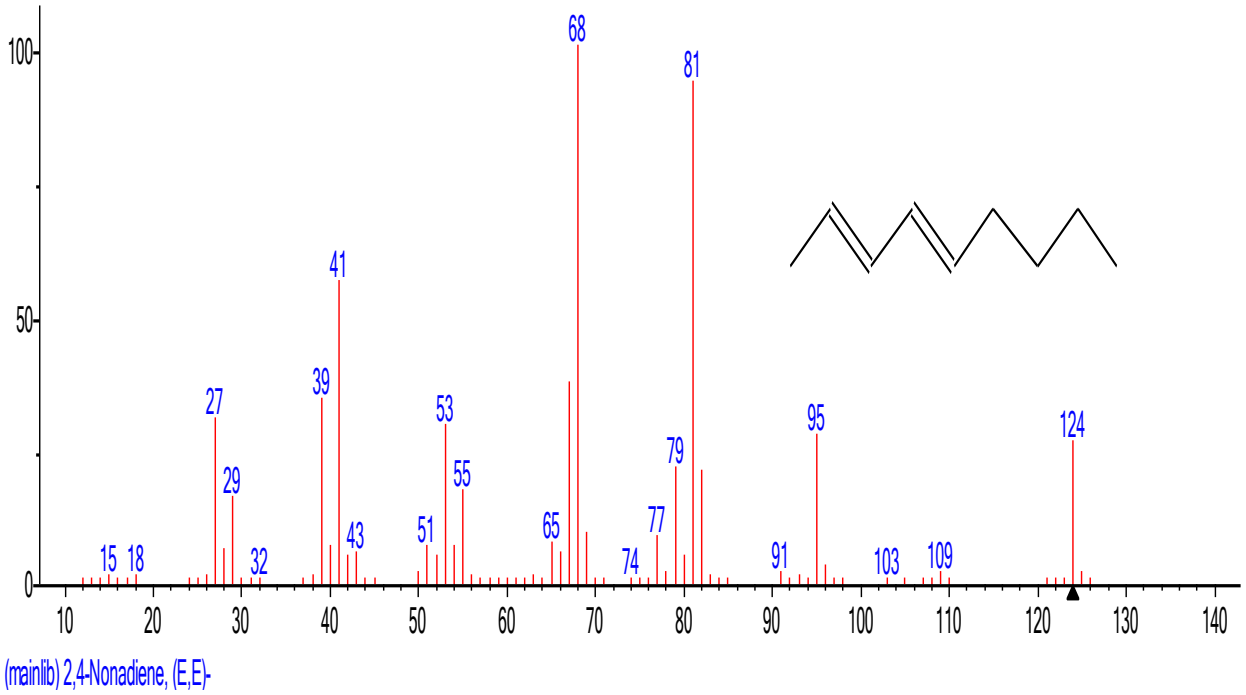
Molecular Weight: 208.38g/mol



Appendix 26: 1-Tridecyne

1-Tridecyne, IUPAC NAME = tridec-1-yne, Molecular Weight: 180.33 g/mol

Activity: H226 (100%): Flammable liquid and vapor [Warning Flammable liquids]

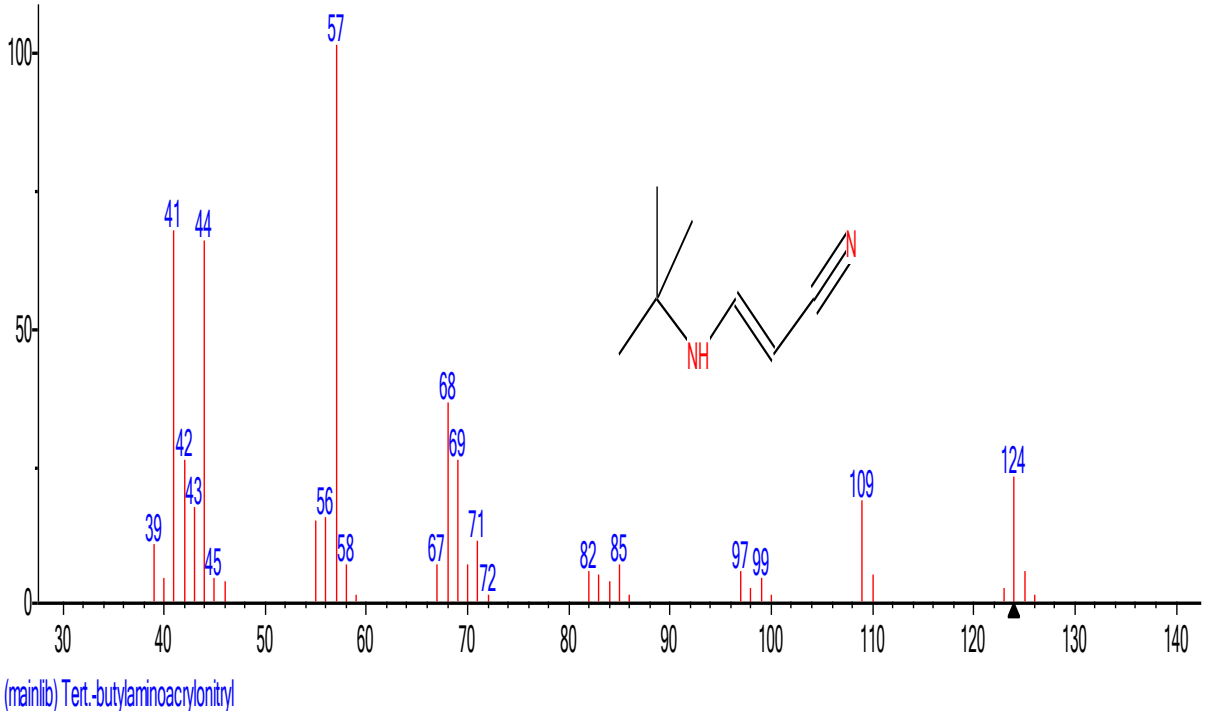


Appendix 27: 2,4-nonadiene, (E,E)-

2,4-nonadiene, (E,E)-, IUPAC NAME = (2E,4E)-nona-2,4-diene,

Molecular Weight: 124.22 g/mol

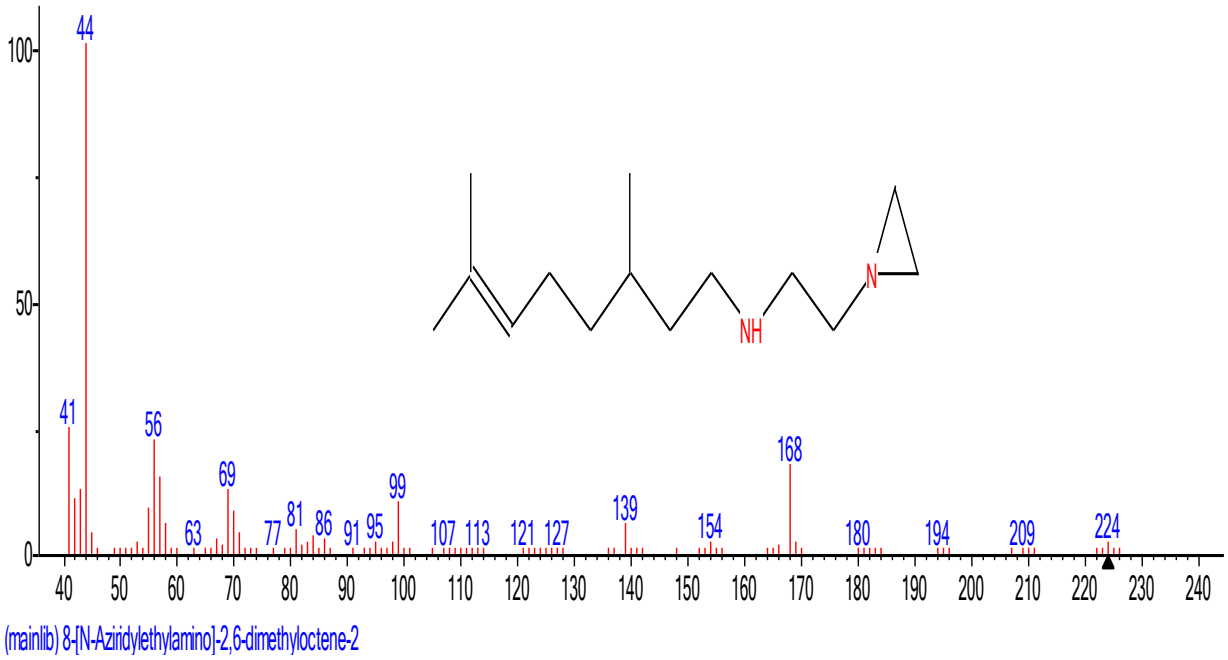
Activity: Flavouring Agent, H226 (100%): Flammable liquid and vapor [Warning Flammable liquids]



Appendix 28: Tert-butylaminoacrylonitryl

Tert-butylaminoacrylonitryl, IUPAC NAME = (E)-3-(tert-butylamino)prop-2-enenitrile

Molecular Weight: 124.18g/mol.



Appendix 29: 8-[N-Aziridylethylamino]-2, 6-dimethyl-oct-2-ene

8-[N-Aziridylethylamino]-2,6-dimethyl-octene-2, IUPAC NAME = (3E,5E)-6,10-dimethylundeca-3,5,9-trien-2-one, Molecular Weight: 192.3g/mol

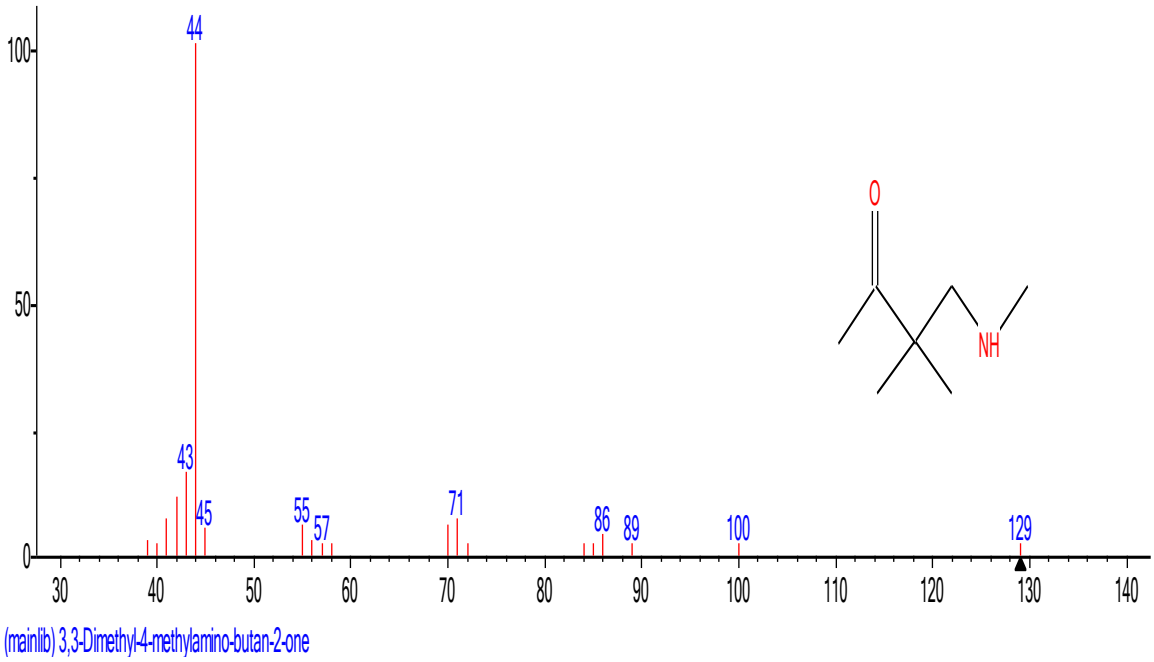
Activity: Flavouring Agent, food additive

H315 (100%): Causes skin irritation [Warning Skin corrosion/irritation]

H317 (100%): May cause an allergic skin reaction [Warning Sensitization, Skin]

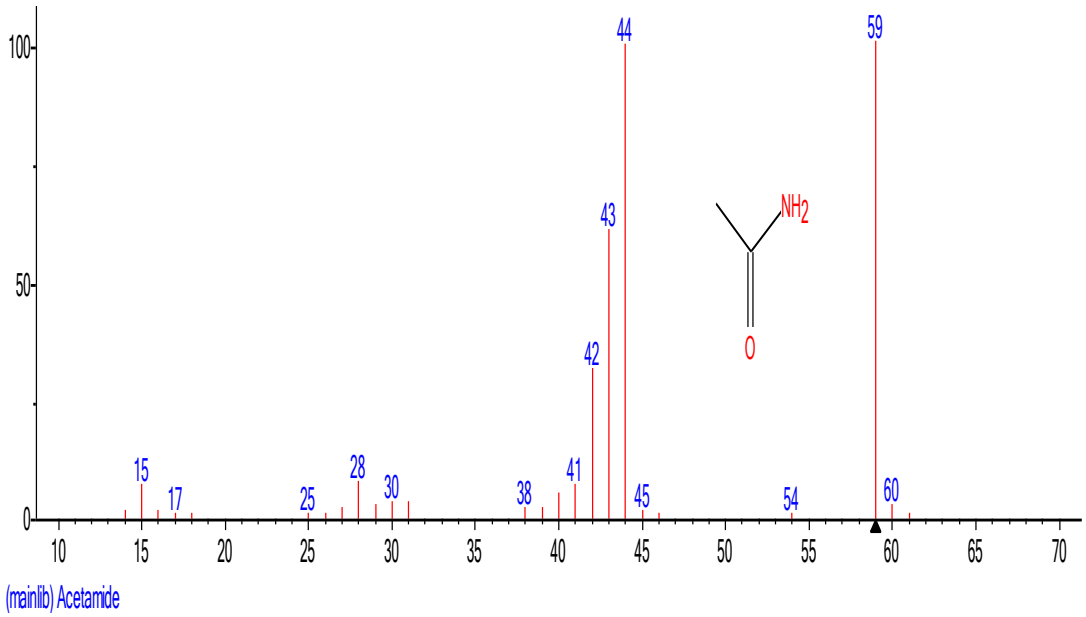
H319 (27.71%): Causes serious eye irritation [Warning Serious eye damage/eye irritation]

H411 (99.71%): Toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment, long-term hazard]



Appendix 30: 3,3-dimethyl-4-methylamino-butan-2-one

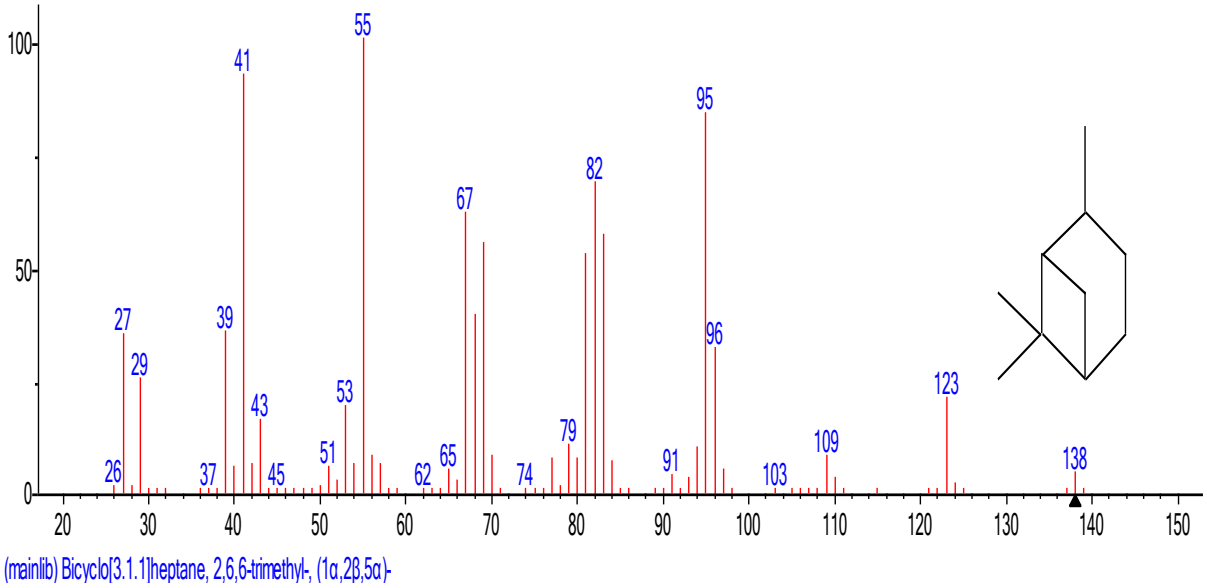
3,3-dimethyl-4-methylamino-butan-2-one, IUPAC NAME = 3,3-dimethyl-4-(methylamino) butan-2-one. Molecular Weight: 129.2 g/mol



Appendix 31: Acetamide

Acetamide, IUPAC NAME = Acetamide, Molecular Weight: 59.07g/mol

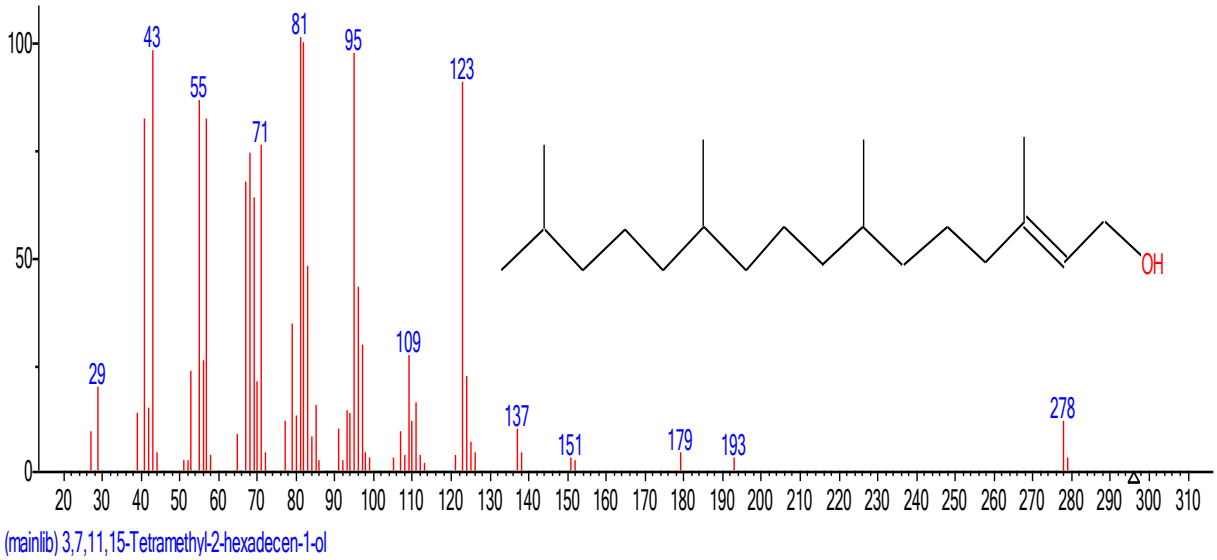
4.4.2 Sample A (*Euphorbia hirta*)



Appendix 32 : Bicycle (3.1.1) heptune

Bicycle (3.1.1) heptune, is flammable and irritant, and the molecular weight is 138.2 g/mol.

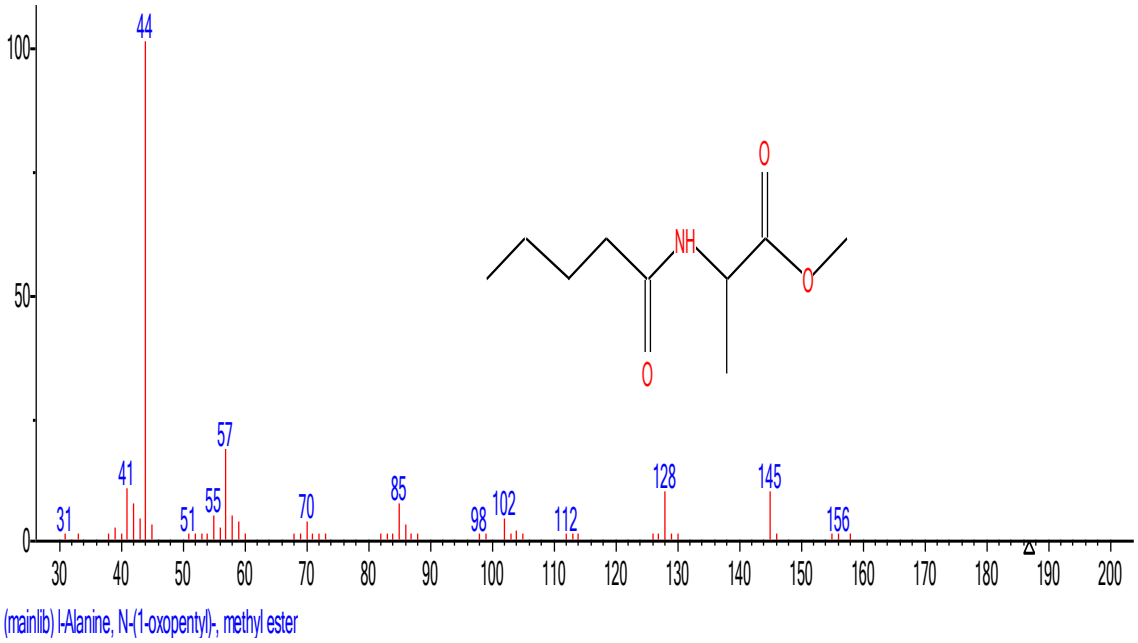
Activity: It irritates skin, eye and respiratory tract. It is toxic on human organ when swallowed.



Appendix 33: 3,7,11,15 tetramethyl-2-hexadecen-1-ol

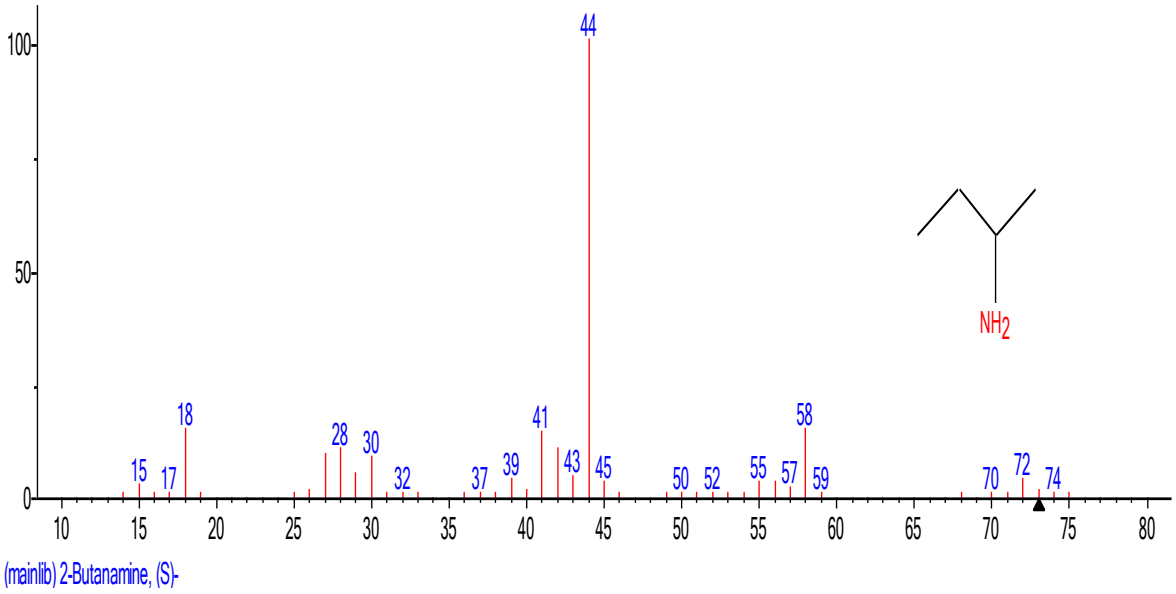
3,7,11,15 tetramethyl-2-hexadecen-1-ol, IUPAC NAME = 3,7,11,15-tetramethylhexadecan-1-ol (Phytol), Molecular Weight: 296.5 g/mol

Activity: It is an acyclic diterpene alcohol and a constituent of chlorophyll. Phytol is commonly used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. Furthermore, phytol also was shown to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR).



Appendix 34: l-Alanine, N-(1-oxopentyl) methyl ester

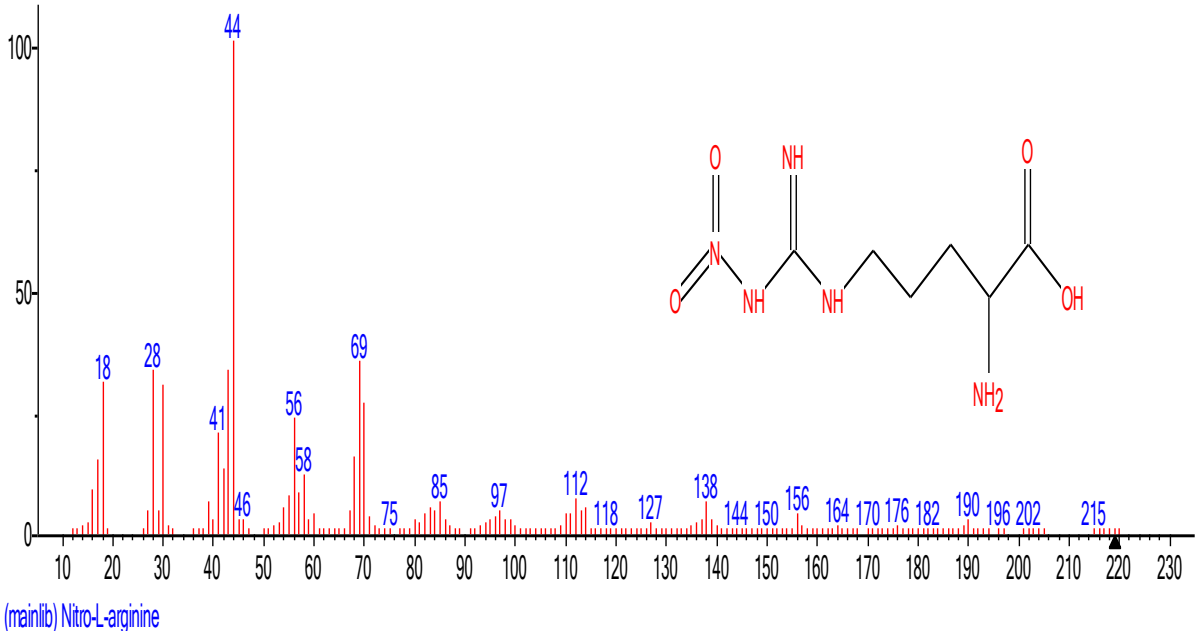
l-Alanine, N-(1-oxopentyl) methyl ester, IUPAC NAME: = Methyl 2-(pentanoylamino) propanoate, Molecular Weight: 187.24 g/mol



Appendix 35: 2-butanamine

2-butanamine, IUPAC NAME = butan-2-amine, Molecular Weight : 73.14 g/mol

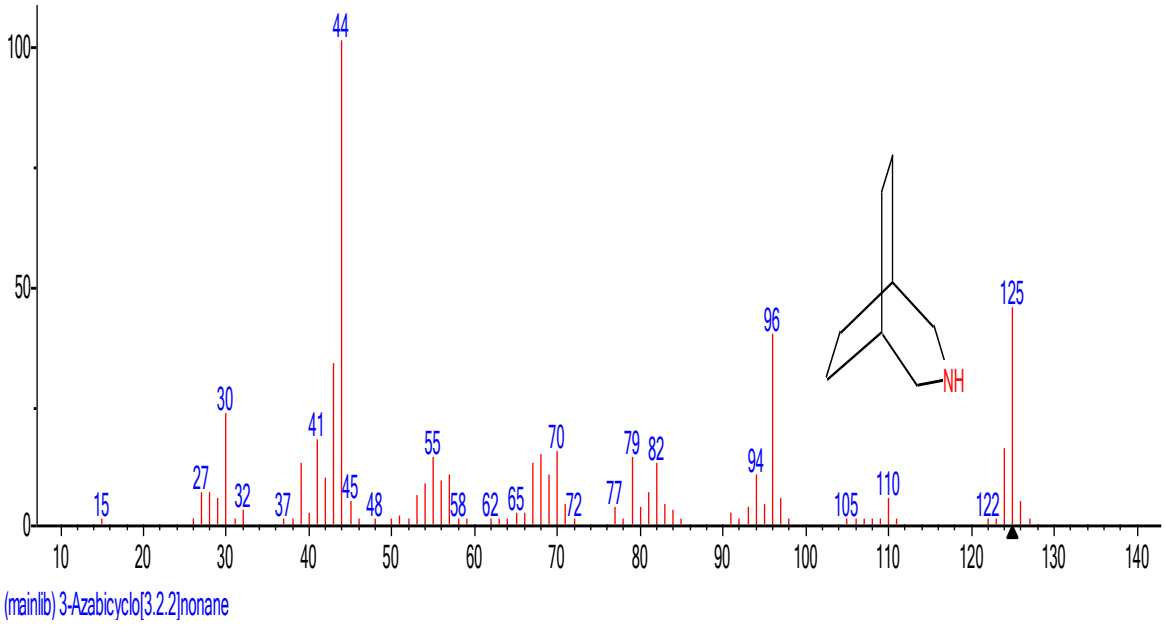
Activity: A fumigant fungicide with a high potential for bioaccumulation, it is not approved for fungicidal use in the European Union. It has a role as an antifungal agrochemical. It is a primary aliphatic amine and an aliphatic nitrogen antifungal agent.



Appendix 36: Nitro-L-arginine

Nitro-L-arginine, IUPAC NAME = Nomega-nitro-L-arginine, Molecular Weight: 219.2 g/mol

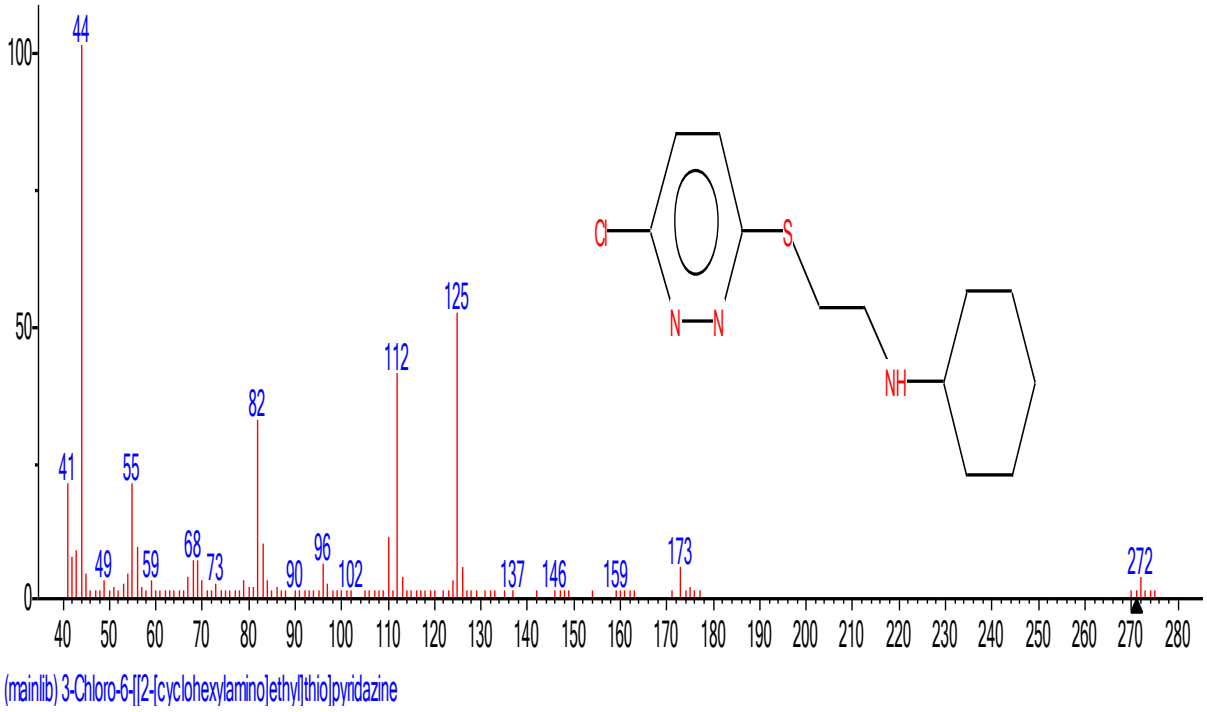
Activity: An inhibitor of nitric oxide synthetase which has been shown to prevent glutamate toxicity. Nitroarginine has been experimentally tested for its ability to prevent ammonia toxicity and ammonia-induced alterations in brain energy and ammonia metabolites



Appendix 37: 3-Azabicyclo(3.2.2)nonane

3-Azabicyclo (3.2.2) nonane. Molecular Weight = 125.21 g/mol,

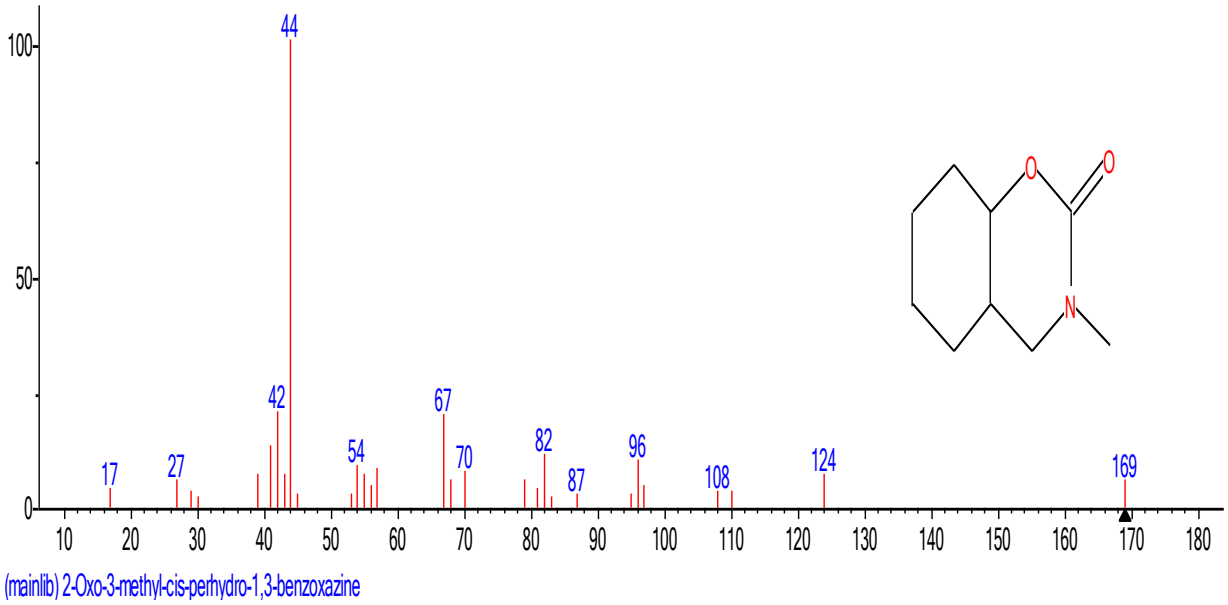
Activity: It has no poisonous effect.



Appendix 38: 3-Chloro-6-[2-(cyclohexylamino) ethyl]pyridazine

3-Chloro-6-[[2-[cyclohexylamino]ethyl] thio] pyridazine

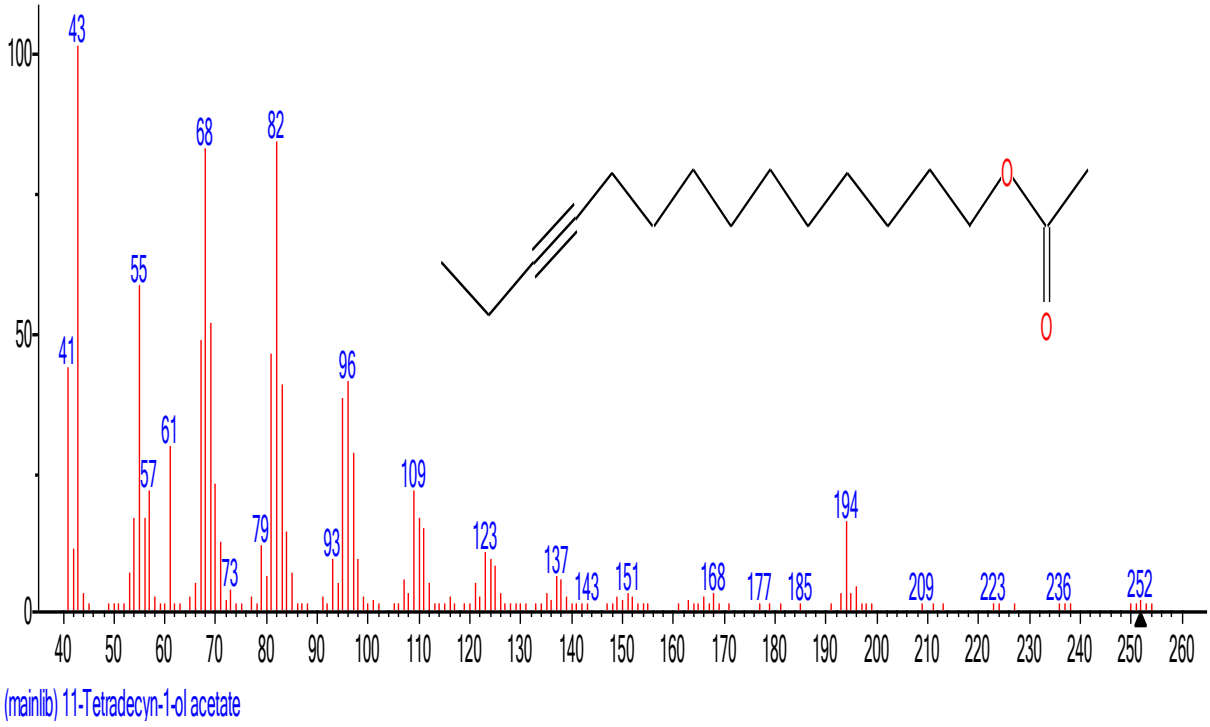
Molecular Weight: 271.81 g/mol



Appendix 39: 2-oxo-3-methyl-cis-perhydro-1,3-benzoxazine

2-oxo-3-methyl-cis-perhydro-1,3-benzoxazine, Molecular Weight: 169.22 g/mol

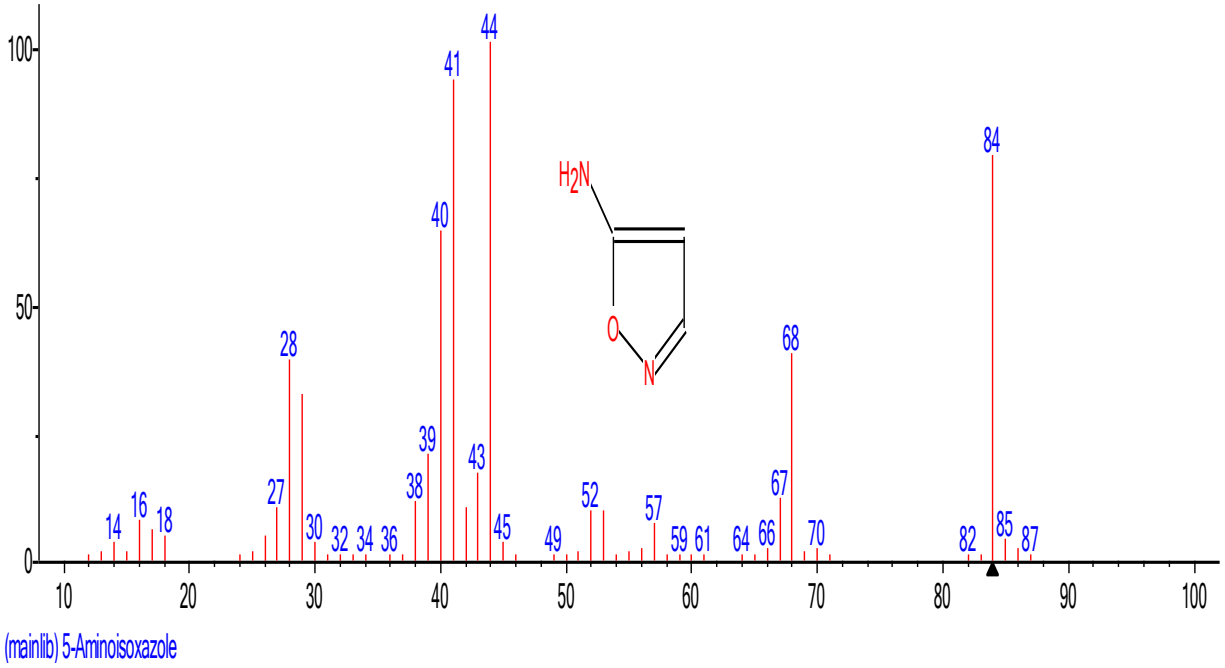
IUPAC NAME = (4*aS*,8*aS*)-3-methyl-4*a*,5,6,7,8,8*a*-hexahydro-4*H*-benzo[e][1,3]oxazin-2-one



Appendix 40: 11-Tetradecyn-1-ol acetate

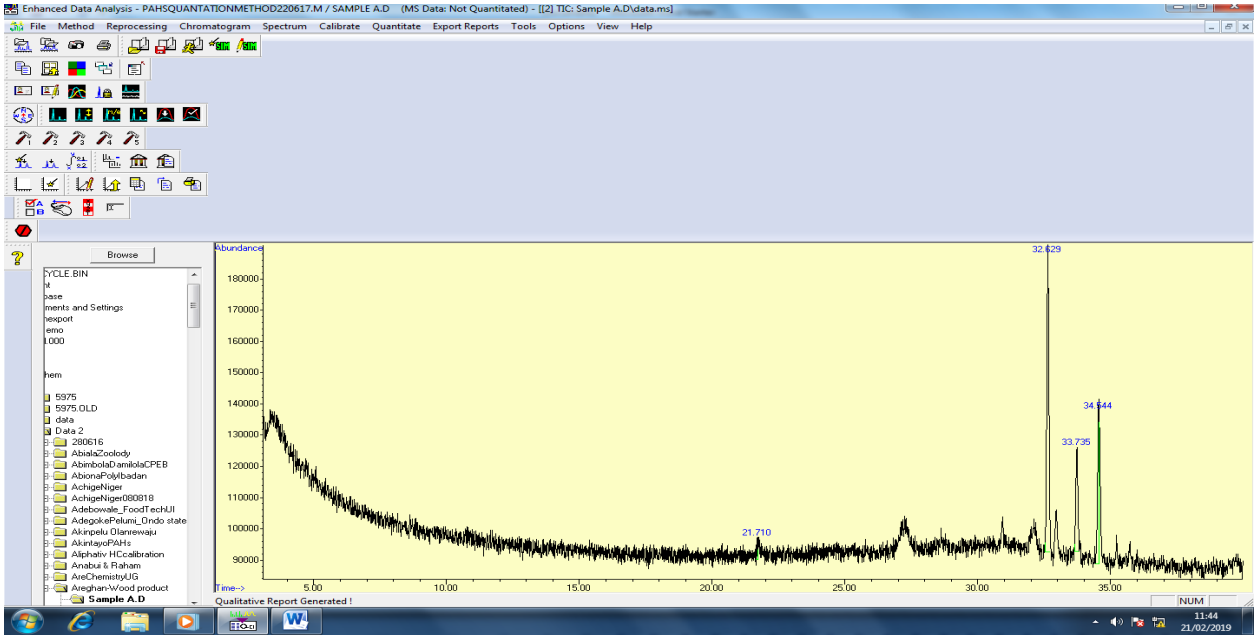
11-Tetradecyn-1-ol acetate ($C_{16}H_{30}O_2$), Molecular Weight: 254.41 g/mol, IUPAC NAME = IUPAC name [(CE)-tetaden 11-enyl] acetate

Activity: It irritates skin, eye and can cause eye damage, respiratory tract irritant. It is toxic on human organs.



Appendix 41: 5-Aminoisoxazole

5-Aminoisoxazole, Molecular weight: 84.08 g/mol, IUPAC NAME = 1, 2-oxazol 5-amine



Appendix 42: Gas Chromatography display