PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES OF *Ensete gilletii* ROOT

EXTRACTS

Ensete gilletii commonly called wild banana, is ethno-medicinally used for the treatment of diarrhea, dysentery, typhoid, stomach pain, kidney stone and liver disorders by herbal practitioners. The dried pulverized roots of the plant were subjected to exhaustive, successive soxhlet extraction using petroleum ether, chloroform and methanol. The result revealed that a higher percentage yield was obtained from the methanol extract (MEG). The GC-MS analysis of the petroleum ether crude (PEG) extract revealed about eighty compounds which are mainly terpenes and hydrocarbons, while the chloroform crude (CEG) extract showed twenty one compounds. The phytochemical screening of the root (PEG, CEG and MEG) extracts revealed the presence of flavonoids, phenols, saponins, tannins, terpenoids, glycosides and sterols. TLC chromatograms show that sub-fraction PEG3 have a better resolved profile with the solvent system; Hex:EtOAc; 5:1 (R_f 0.45, 0.7). A compound was isolated from sub-fraction PEG3c; the isolated compound (PEG3c1) was characterized using physical, chemical and spectroscopic techniques. The antibacterial activities of the extracts were evaluated by the disc diffusion method against selected organisms, in comparison with standard reference (ampicillin). The extracts were active against *Salmonella typhii, Escherichia coli, Klebsilla pneumoniae, Shigella dysentriae* and *Staphylococcus aureus* with zones of inhibition ranging from 16 mm to 28 mm when compared to a standard drug. MIC and MBC values were 40 mg/cm^3 and 80 mg/cm^3 respectively. The results confirmed the ethno-medicinal claims on the plant and could be used as a lead to the discovery of potent medicaments.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

1.1.1 Importance of plants

Plants are living organisms of the kind exemplified by trees, shrubs, grasses, vines, ferns and mosses, usually absorbing water, and inorganic nutrients through its roots (Merriam-Webster, 2009). They are either wild plant species or domesticated wild species that grow spontaneously in self-maintaining populations, natural or semi-natural ecosystems and could exist independently of constant human activity. Domesticated plants species are those that spring up through human actions such as selection or breeding and depend on management for their occurrence (Calixto, 2000). Green plants obtain most of their energy and food from sunlight through the process of photosynthesis by primary chloroplasts that are derived from endosymbiosis with cyanobacteria. The leaves of plants vary in shapes, colour and size (Ahn, 2017). Green plants provide a significant proportion of the world's molecular oxygen and are the basis of most of earth's ecosystems, particularly on land.

Plants yield grains, tubers, fruits and vegetables, form essential foods for both humans and animals and have been made a part of domestic use over the years. Plants have many cultural uses and are also used as ornaments, building materials, writing materials and in great variety, they have been the source of medicines and drugs (Samuelsson, 2004). They produce oxygen in exchange of carbon (IV) oxide, and aid in air purification and also prevent direct ultra-violet rays from reaching the earth surface. They are the primary habitat for thousands of animals and microorganisms. Plants help moderate the temperature and generate shelter from sun, rain, storm and wind. Many species of plants are explored locally as a source of traditional herbs and in certain spiritual activities (Summer, 2000). They are also important in synthesis of modern drugs.

1.1.2 Medicinal uses of plants

A medicinal plant is a plant which one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical semi-synthesis (WHO, 2005). For centuries, plants have been used for medicinal purposes (Samuelsson, 2004). In recent years, the use of plants as a source of medicine has become an increasingly incorporated into scientifically based system of healing. The increasing demand on natural products has improved studies into the scientific basis and therapeutic abilities of plants, allowing herbal practitioners gain acceptance among the medical sector (Karunamoorth *et al.,* 2012). The medicinal uses of plants were formulated through observations of animals and by trial, error, failure and eventually successful (RamGopal, 2006). Medicinal plants are regarded as rich sources of components which can be used in drug manufacturing. In recent time, plants are found in diverse medicinal applications ranging from; the production of medicines to cosmetics, herbal drinks and food supplements and other items in daily uses. Herbs are found to have significant role particularly in modern time industries. The system is based on the belief that plants have the vast ability for curing and improving health when used as medicine and as herbal food products (Goyal, 2008; Rajakumar, 2014). The therapeutic properties of plants could be based on their anti-oxidant, anti-microbial, anti-pyretic, anti-inflammatory and anti-diabetic effects of the phytochemical constituents present in them (Adesokan *et al.,* 2008).

 Hasler (2005), had earlier stated that herbs which have supporting, enhancing or curative potentials should be added to food products in order to improve their medicinal effects. Scientific value of plants is on the rise, resulting from microbes such as; virus, bacteria and fungus originated

diseases which are widespread and most organisms have developed resistance against synthetic drugs, thereby increasing the need for a more effective, reliable and natural remedy (Gurib-Fakim, 2006; WHO, 2018). According to World Health Organization, medicinal plants would be the greatest source for obtaining an array of drugs. Therefore, potentially active plants should be studied to better understanding of their properties, safety practices in addition to usefulness (Nascimento *et al.,* 2013).

Most plants are regarded as important source of nutrition and soothing effect, as a result, they are recommended for their therapeutic values. Some of these plants include; ginger, green tea, garlic, cinnamon, walnut, aloe vera and turmeric to mention a few. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin, toothpaste, mouth-wash, body-wash, soap and in other herbal products formulation (Tarbuti *et al.,* 2003). According to several documented reports, many drugs listed as conventional medications were originally obtained from plants. Medicinal plant therapy is based on the empirical findings of hundreds of years of use. Staniszewska *et al.* (2003) reported that *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species and *Papaver somniferum* (poppy juice), among others are used for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. The interest in nature as a source of potential chemotherapeutic agents by both local and foreign researchers is on the increase. Cragg and Newmann (2005) had earlier recorded that 50% of drugs in clinical use were produced from natural products and their derivatives.

Plants synthesize hundreds of chemical compounds for several functions, including; defense against insects, fungi, diseases and herbivorous mammals (Tapsell *et al*., 2006). Several phytochemical constituents with potentials or confirmed biological activities have been identified

over the years, mainly through ethno-botanical studies. Medicinal plants are widely used in non-industrialized societies and rural communities, mainly because they are readily available and cheaper with few side effects compared to modern synthetic medicines. In Africa, the practice of herbal medicine is well known and established; as a result, most of the plants that are used for medicinal and other purposes come from rural areas due to the existing knowledge of herbalist. Majority of the claims by local practitioners have been proven to be true, while; some are still under review. The annual global export value of 50,000 to 70,000 types of plants suspected to contain medicinal properties was estimated to be 2.2 billion US$ in 2012, while in 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars (Ahn, 2017; Medicinal and Aromatic, 2017).

1.1.3 Plant constituents

Plants produce a vast number of chemical compounds that are known as secondary metabolites /natural products/phytochemicals/phytoconstituents/bioactive compounds. Secondary metabolites are the chemicals that are not required for the direct survival of plant, but are synthesized to increase adaptation of the plant by protecting it from pathogens, herbivores, insects and other environmental factors (Kennedy and Wightman, 2015; Shalini and Shampathkumar, 2012). Phytochemicals accumulate in different parts of plant, such as; the root, stem, bark, leaves, fruits and seeds. Plant natural products belong to various classes of compounds, including; isoprenoids, phenylpropanoids, triterpenoids, alkaloids, glycosides, tannins, flavonoids, saponins, essential oils and other related constituents which exert physiological actions in addition to the carbohydrates, proteins and lipids that are often found as conjugated products of compounds derived from

different biosynthetic pathways (Gurib-Fakim, 2006). Most of plant secondary metabolites are therapeutically active, while others are inactive. In recent time, the active constituents responsible for medicinal actions of plants have been screened, studied, observed, isolated and characterized. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those ascribed to macronutrients and micronutrients (Azene *et al.,* 2016). Several plants have been reported to contain phytochemicals which are responsible for their medicinal values. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. *Cinchona* bark is the source of malaria-fighting quinine (Manuchair, 2002). It was recorded that most of the major anticancer drugs are natural products, either from plants or micro-organisms, such as; Bleomycin, Doxorubicin, Vincristine, Vinblastine paclitaxel (Taxol), Ironotecan (a camptothecin derivative), Etoposide and Tenoposide (Manuchair, 2002; Gurib-Fakim, 2006). Herbal researchers believe that plant medicinal effects in general are the sum of their constituents and cannot be reduced to just a specific compound alone. Researches has proven that medicinal plants contain chemicals that have been sub-divided into fourteen main groups; alkaloids, anthocyanins, anthraquinones, cardiac glycosides, coumarins, cynogenic glycosides, flavonoids, glucosilinates, minerals, phenols, saponins, tannins, vitamins and volatile oils (Taiz and Zeiger, 2006). The active plant constituents are usually classified by their chemical structure rather than their effect.

Even though compounds found in plants are of many kinds but most are in four major bio-chemical classes; alkaloids, glycosides, polyphenols and terpenes (Kennedy and Wightman, 2015).

1.1.4 Preliminary extraction of phytochemicals using different methods

Extraction is the separation of medicinally active constituents of plant tissues using selective solvents through standard procedures. The extracts obtained from plants are relatively complex mixtures of metabolites which are usually in liquid, semisolid, solid state or in dry powder form, after evaporation of the solvent (Prashant *et al.,* 2011). An important factor that controls the choice of solvents used in an extraction is the type of phytochemical constituents that are to be extracted (Handa *et al.,* 2008). Several methods adopted for plant extraction include; maceration, infusion, percolation, digestion, decoction, hot continuous extraction (soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction, fragrance extraction) may be employed. Some of the recent extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation (Bimakr, 2010; Handa *et al.,* 2008; Prashant *et al.,* 2011). Most extraction methods can be direct, which involves bringing the plant material in contact with the solvent for a period of time; or serial which involves the use of many solvents of varying polarities successively on the same material. Soxhlet extractor can be very useful for the exhaustive and successive extraction of plant constituents with various solvents, but this cannot be used for thermolabile or degradable compounds. This challenge may be overcome by extracting under reduced pressure and temperature (Ncube *et al.,* 2008; Nikhal *et al*., 2010). Several researchers have used various organic and inorganic solvents while extracting constituents from plant samples.

1.2 Statement of the Research Problem

The spread of diseases caused by microorganisms is a major concern in the pharmaceutical world as thousands of people suffer from persistent microbes originated ailments, owing to the fast increase of bacteria resistance to the common antibiotics, which has raised the need for alternative active compounds from plants in order to treat the bacterial infections (Akinpelu *et al.,* 2008; WHO, 2018). Resistance to medications are emerging and spreading globally, threatening the use of synthetic drugs in the treatment of common bacterial infectious diseases, resulting into prolonged illness, disability and sometimes death. The use of synthetic drugs, though effective is often associated with side effects which cannot be ignored as there is a continuous increase in reported relapse resulting from the synthesized components. Synthetic drugs can be relatively expensive, rare in local communities in comparison with natural products; such as plants (Karunamoorth *et al.,* 2012).

1.3 Justification of the Study

Studies have shown that natural products are sources of potent antibacterial drugs and many researchers are compelled to further investigate the biological activities of various medicinal plants. The use of herbal medicines for the treatment of infections is popular, especially in rural communities due to their availability, effectiveness, little or no side effects.

The ever increasing need for the discovery of new and effective means for the control and cure of various ailments has become a global concern, so that the search for natural cure and control of diseases have increased. The roots of *Ensete gilletii* is traditionally used for the treatment of bacterial infections, such as; diarrhea, dysentery, stomach ache and infertility. Extensive literature

search have revealed no much information on the isolation and characterisation of phytochemical constituents as well as investigation of the ethno-medicinal antibacterial claim on roots of *Ensete gilletii.* Therefore, this study was undertaken.

1.4 Boundaries of the Study

The present study focuses on sequential extraction of phytochemicals of *Ensete gilletii* roots using three different organic solvents; petroleum ether, chloroform and methanol. The phytochemical constituents of the three extracts will be screened, for their antibacterial potentials.

1.5 Aim and Objectives of the Study

Aim

This research work is focused on the phytochemical, characterisation of chemical constituents and antibacterial studies of *Ensete gilletii* root extracts.

Objectives

In order to achieve the aim of this study, the following objectives were undertaken:

i. Quantitative screening of the pulverized sample using standard procedures.

ii. Successive extraction of phytochemicals from the dried roots of *Ensete gilletii* using petroleum ether, chloroform and methanol by the continuous extraction (soxhlet) method.

iii. Preliminary antibacterial screening of the crude extracts using standard methods.

iv. Qualitative screening of the crude extracts using standard methods.

v. Isolation and purification of one or more phytochemical(s) from the extract with promising spots using chromatographic techniques.

vi. Structural elucidation of the isolated compound(s) using physical, chemical and spectroscopic techniques.

CHAPTER TWO

2.0   **LITERATURE REVIEW**

2.1 *Ensete gilletii*

2.1.1 Taxonomy

Kingdom: *Plantae*

Division: Angiosperms

Class: Monocots

Order: Zingiberales

Family: *Musaceae*

Genus: *Ensete*

Specie: *Ensete gilletii*

Synonym: *Ensete livngstonianum*

(Wu *et al.,* 2015).

Local names: Wild banana (English), Ayabardaji (Hausa), Uhiaunune (Igbo), Egboogede (Yoruba).

2.1.2 Description and occurrence

Ensete gilletii commonly called "wild banana" (De Wild), is a large monocarpic unbranched herbs, having a wide-spreading and immensely long paddle-shaped leaves with usually crimson midribs, it rarely sucker and die after flowering. The leafy young stem dies and become a hard round corm about 6 cm tall, while remaining on the surface of the soil. The fertile stem nodes, bearing a mass of small fruits about 2cm long, have yellow or orange pulp and large dark brown seeds (Bekele and Shigeta, 2011). The roots are fibrous and grow deep into the soil.

Plate 2.1: *Ensete gilletii* **whole plant**

Generally, the *Ensete* is a genus of monocarpic, flowering plants that grows in tropical regions of Africa and Asia. It is one of the three genera in the *Musaceae* family (Baker & Simmonds, 1953).

The genus *Ensete* consists of nine species, namely*; Ensete gilletii, Ensete ventricosum, Ensete superbum, Ensete homblei, Ensete perrieri, Ensete glaucum, Ensete lasiocarpum, Ensete wilsonii, Ensete sp*. and it is distributed in tropical regions of Africa and Southern Asia. Among these species, *Ensete gilletii* is found in Nigeria, Cameroon, Sierra Leone and Angola while *Ensete ventricosum* is cultivated in Ethiopia. *Ensete homblei* is native to Congo and Zambia while *Ensete superbum and Ensete glaucum* are reported to occur in India, *Ensete perrieri* is reported to come

from Madagascar (Simmonds, 1960). Amongst the species of *Ensete*; *Ensete ventricosum* is widely spread in Ethiopia and it is the most investigated specie of *Ensete* (Diro and Staden, 2005).

2.1.3 Ethno-medicinal uses

Ensete gilletii was earlier listed as one of the rich but unexplored medicinal plants in Africa (Vasundharan *et al.,* 2013). *Ensete gilletii* is used ethno-medicinally in the treatment of various ailments such as; stomach ache, diarrhea, dysentery, pneumonia, typhoid, kidney stone and liver disorder (Afolayan *et al.,* 2014; Bekele and Shigeta, 2011). Other species of *Ensete,* such as; *Ensete ventricosum and Ensete glaucum* are exploited as traditional medicines. *Ensete ventricosum* is useful in the treatment of ailments including; stomach ache, menstrual pains, diarrhea, dysentery, liver disorder among others (Afolayan *et al*., 2014; Azene *et al*., 2016; Sethiya *et al*., 2016). In Ethiopia, decoction of leaves of *E.ventricosum* is used in the treatment of broken bones, stimulation of labour or induce abortion. Also, hepatitis and other liver grievance are treated with ash and infusions from the fruit and leaves of *E.ventricosum,* the seeds facilitate the discharge of placentas in both human and animals (Tarbuti *et al.,* 2003; SarojKumar *et al.,* 2015). The seeds of *Ensete Superbum* are used to treat wide range of human diseases, which includes; appendicitis, cancer, diabetes, leucorrhoea, kidney stone, dog bite, dysuria, measles, psychosomatic disorder among others (SarojKumar *et al*., 2015), while the roots are used for stomach ache, easy delivery, semen production birth control among others, the leaves and fruits are used to raise uterus (Jagtap *et al,* 2008).

2.1.4 Non ethno-medicinal uses

Ensete gilletii is generally used as a source of food in parts of Africa where it is cultivated. The plant produces banana-like fruits which are seedy and mostly not edible, but parts of the plant are

consumed as food. The under-ground corm and the aerial pseudostem made up of overlapping leaf sheaths are edible (Gebre and Nikolayev, 1993). In Ethiopia, the fermented chopped and grated pulp of the corms and leaf sheaths are used as flour in making kocho bread (SarojKumar *et al.,* 2015). The corm is cooked and eaten in a similar way as potato. The pulverized corm or scraped pseudostem is used to produce starchy food after fermentation process (Birmeta *et al.,* 2004). The leaf sheaths provide good quality fiber for making rope, baskets, mats and sacks. The dried leaf sheaths are used in fences and house construction. Fresh leaves provide shades in nurseries and also used in feeding livestock (Tobiaw and Bekele, 2011).

2.2 Phytochemistry and Biological Activity of the *Musaceae* (banana) Family

Banana is one of the most important fruit crops of several countries due to its enriched food quality and wide range of medicinal value. Fruits, leaves, peels, root, and stalks from banana plants have been used as a source of medicine for the treatment of several ailments including; diarrhea and dysentery, in the healing of intestinal lesions in colitis (Stover and Simmonds, 1987), antilithic activity (Prasad *et al.,* 1993), inflammation, pains and snakebite (Houghton and Skari, 1992), antiulcerogenic activity (Lewis *et al.,* 1999; Goel and Sairam, 2002), hypoglycemic effect (Eseyin *et al.,* 2011; Wu *et al.,* 2015), and hypolipidemic and antioxidant actions (Krishnan and Vijayalakshmi, 2005). Phytochemical studies revealed that natural products found in banana family are derived from various biosynthetic pathways, including; terpenoids (Taiz and Zeiger, 2006) and phenyl-propanoids, such as; flavonoids (Lewis *et al.,* 1999; Pascual-Villalobos and Rodriguez, 2007).

Various preparations of dried unripe banana were used in aspirin- induced ulcerations in rats, the result showed anti-ulcerogenic activity and were effective both as a prophylactic treatment and in

healing ulcers already induced by aspirin. It was reported that the antiulcerogenic action of banana appeared to be due to its stability to stimulate the growth of gastric mucosa (Best *et al.,* 1984).

Banana flakes examined against diarrhea was observed to reduce the severity of diarrhea in critically ill tube-fed patients. It was concluded that banana flakes can be used as a safe, cost-effective treatment for diarrhea (Emery *et al.,* 1997). Banana extracts was reported to show significant antibacterial activity (Imam *et al.,* 2011; Ono *et al.,* 1998). Other reported activities include; wound healing (Agarwal *et al.,* 2009), anti-allergic activity (Tewtrakul *et al.,* 2008). The antivenom action of the stem juice from banana plant was also reported (Houghton and Skari 1992; Borges *et al.,* 2005).

Dried peels, pulps and seeds of banana fruits were studied *in-vitro* for their antibacterial and antioxidant activities, the results showed significant activity (Jain *et al.,* 2011; Tan *et al.,* 2012). Sampath *et al.* (2012), reported that all parts of the banana plant have medicinal applications, also, banana lectins exhibit the potential of inhibiting HIV-1 reverse transcriptase activity, suppressing cancer cell proliferation and stimulating macrophage activities. Similarly, ripened and unrippened fruit pulp of *Musa paradisiaca* was studied, the result displayed the presence of antibiotics which could be used as an effective antimicrobial agent (Jyothirmayi *et al.,* 2011). Mutagenic effect in the peripheral blood cells of Swiss albino mice induced with *Musa paradisiaca* fruit peel extract was observed to have a significant increase in the average numbers of DNA damage in peripheral blood leukocytes (Andrade *et al.,* 2008).

Five *Bacillus stearothermophilus* α-glucosidase inhibitors which were isolated from the flowers of *Musa spp*, were studied. It was reported that the isolates exhibited strong α-glucosidase inhibitory effect. The result proved that *Musa spp* flowers could be used as a source of effective

antidiabetis (Dai *et al.,* 2014). The isolates were identified as; vanillic acid **(I),** ferulic acid **(II),** β-sitosterol **(III),** 9-(4-hydroxyphenyl)-2-methoxyphenalen-1-one **(IV)** and daucosterol **(V)** (Dai *et al.,* 2014).
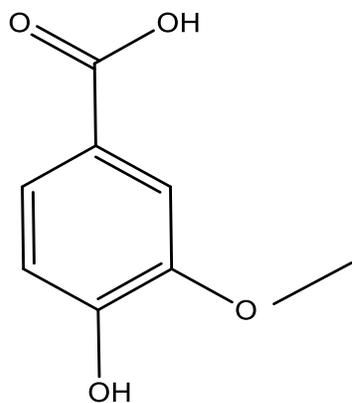
(I)
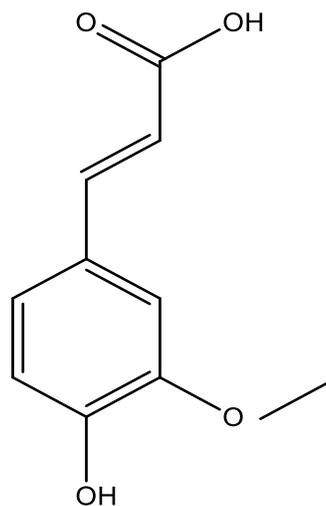
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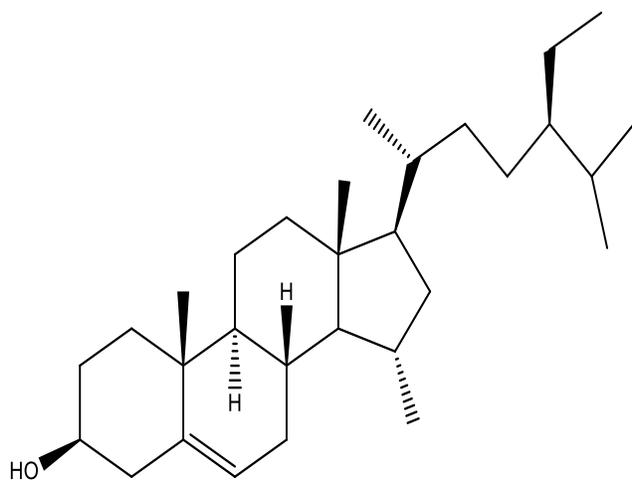
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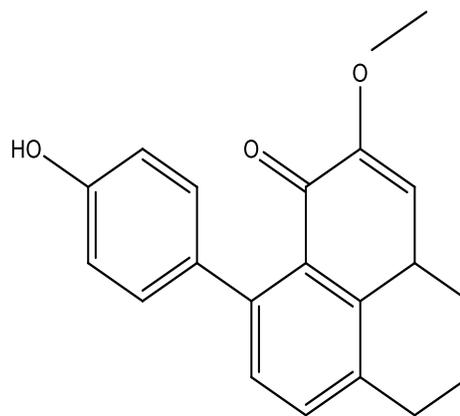
(IV)
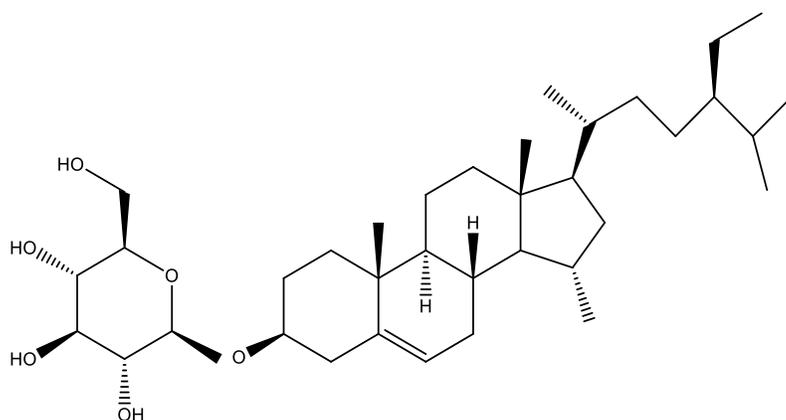
(V)

A constituent hydroxyanigorufone **(VI)** obtained from *Musa paradisiaca* showed to be a potential cancer chemopreventive agent (Jang *et al.,* 2002).
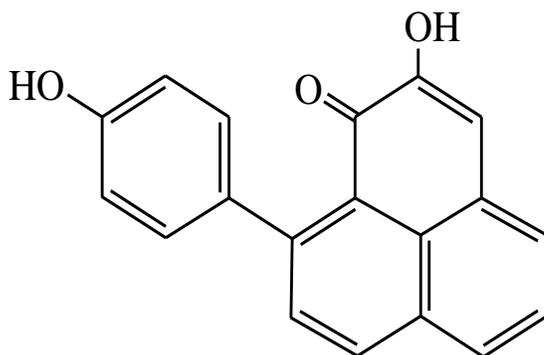
(VI)

Phenylphenalenones derived from the phenylpropanoid biosynthetic pathway were reported as secondary plant metabolites produced in response to pathogen attacks (Luis *et al.,* 1996; Kamo *et al.*, 2001). Diarylheptanoids **(VII)** and phenylphenalenones are derived from the phenylpropanoid biosynthetic pathway. Diarylheptanoids possess two aryl rings linked with a chain of seven carbon atoms.
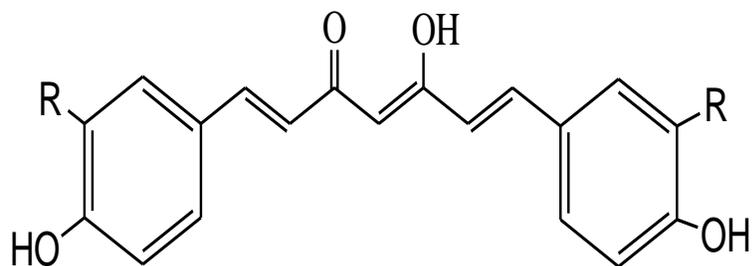
**(VII)**   R= H or R= OCH$_3$

Curcumin **(VIII),** the most studied diarylheptanoid compound isolated from *Musa paradisiaca* is used as a health-promoting substance (Del Rio *et al*., 2006).
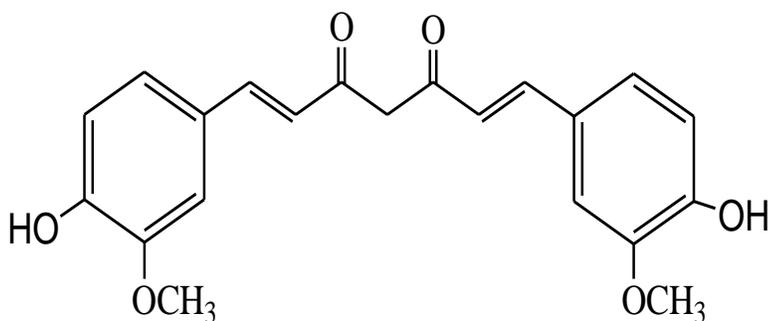
(VIII)

Their chemical structure consists of a tricyclic phenalene nucleus, ketone groups (on ring A or B) and a lateral phenyl substituent. Phenylphenalenones can be categorized into two groups according to oxidation patterns on the tricyclic phenalene nucleus (Otálvaro *et al.*, 2007). In *Musaceae,* mainly phenylphenalenones **(IX)** bearing oxygenated functional groups on the first ring were found. Phenylphenalenones and diarylheptanoid-related structures isolated from *Musa sp* **(X)**.

R$_1$ = OH, R$_2$ = OCH$_3$
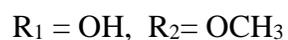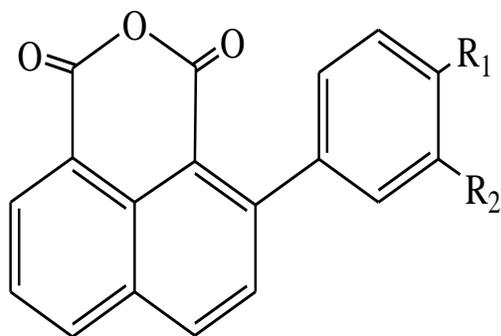
(IX)
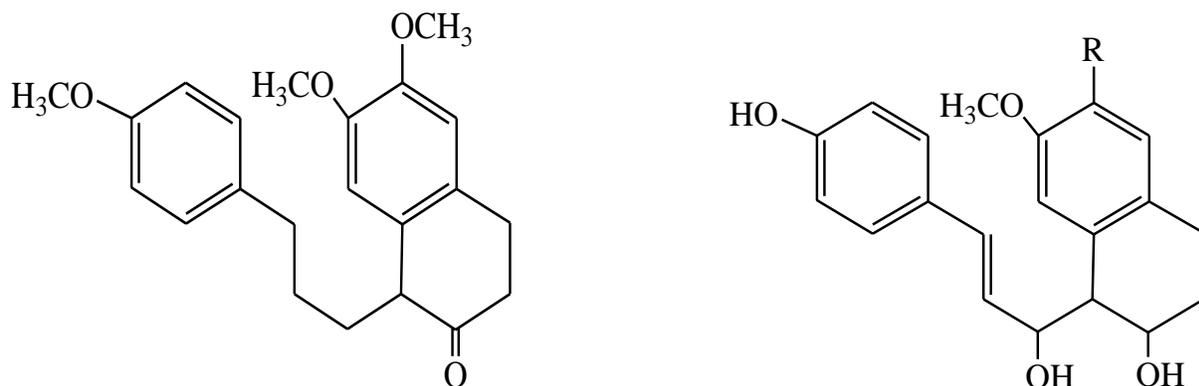
(X)

It was documented that 9-Phenylphenalenones **(XI)** were found in all three genera, *Musella* (Qin *et al.*, 2006)*, Ensete* (Holscher and Schneider, 1998) and *Musa* (Luis *et al.*, 1996; Kamo *et al.*, 2001; Jang *et al.*, 2002; Del Rio *et al*., 2006; Otálvaro *et al.*, 2007). In addition, 4-phenylphenalenones **(XII)**, were mainly found in the genus *Musa* alongside with 2-phenyl-1, 8-naphthalic anhydrides (Kamo *et al.*, 2001).
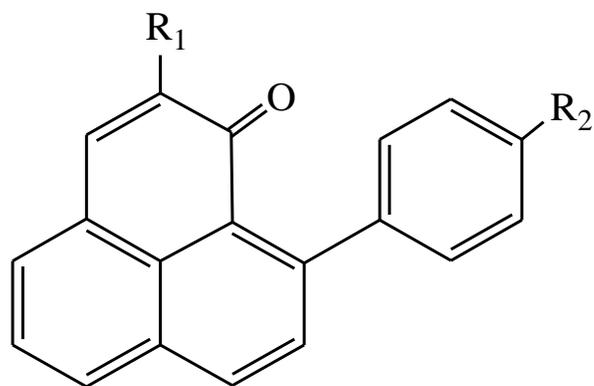
(XI)
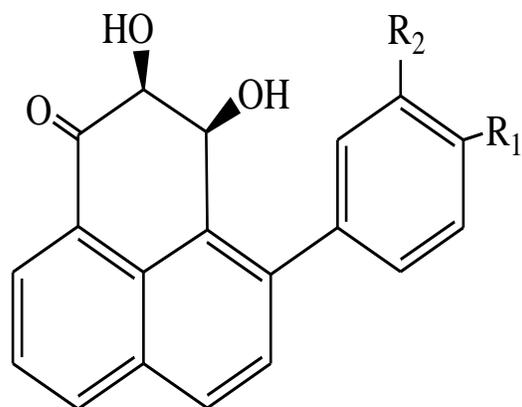
$R_1 = OH$, $R_2 = OCH_3$ or H

(XII)

Oxabenzochry-senone **(XIV)** was isolated from *Musa acuminata* (Opitz *et al.*, 2002).
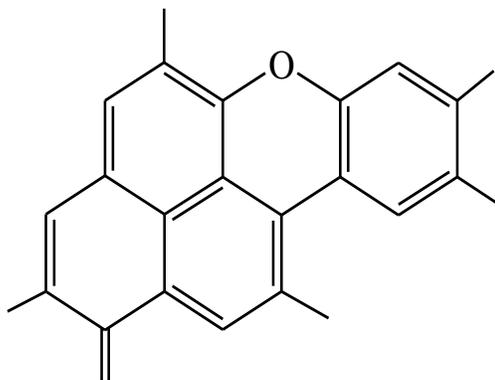
(**XIV**)

Lectin was first isolated and identified from *Musa paradisiaca* (Koshte *et al*., 1990; 1992). Several others were isolated and studied from *Musa acuminate* and *Musa spp* (Clendennen and May, 1997; Peumans *et al.,* 2000; Meagher *et al.,* 2005). It was observed that the banana lectin was abundant in the roots and pulp than in any other part of the plant (Sanjenbam *et al.,* 2014).

An active antiulcerogenic ingredient was extracted from unripe banana by solvent fractionation and it was identified as the flavonoid leucocyanidin (**XV**) (Lewis *et al*., 1999).
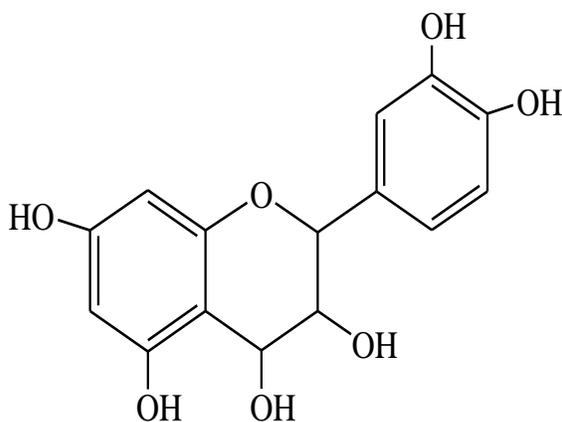
(**XV**)

Dopamine serotonin, norepinephrine, tryptophan, indole compounds, alkaloids, tannin, ascorbic acid, several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) have been isolated from the pulp of different banana species. Sterols such as β-sitosterol **(XVI)**, campesterol, stigmasterol were isolated from the fruit peel of banana (Mokbel and Hashinaga, 2005).
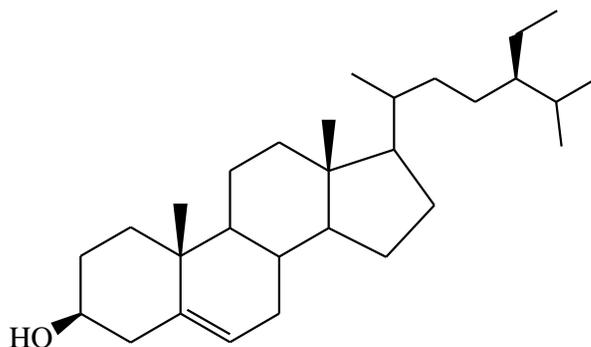
(**XVI**)

The presence of flavonoids and other phytochemicals increases the antioxidant potential of different parts of banana.

2.3 Phytochemistry

2.3.1 *Ensete gilletii*

Phytochemical study of the seeds of *Ensete gilletii* fruits, were found to contain secondary metabolites such as: alkaloids, flavonoids, phenols, steroids and tannins (Afolayan *et al.,* 2014). Literature reveals no much work carried out on *Ensete gilletii* in terms of isolation and characterization of its bio-actives. However, researches on the phytochemical constituents of other species in the *Ensete* genus have been reported.

2.3.2 *Ensete ventricosum*

Phytochemical analysis of *Ensete ventricosum* seeds revealed the presence of alkaloids, steroids, phenolics, glycosides and sugars (Azene *et al.,* 2016; Vasundharan *et al.,* 2013). Earlier, Holscher and Schneider (1998) isolated and characterized phenylphenalenone **(XVII)** from *E.ventricosum.*
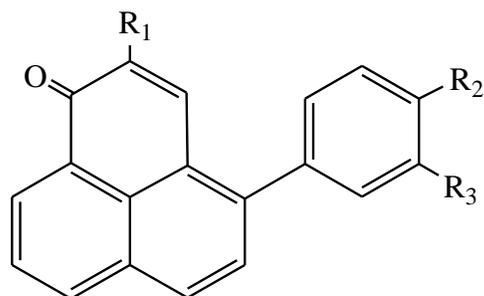
$R_1, R_2, R_3 = H$ or $R_1, R_2, R_3 = CH_3$

(XVII)

A chromatin derivative which contains no steroidal phytosterol was isolated from seeds of *E.ventricosum* (Sethiya *et al.,* 2016).

2.3.3 *Ensete superbum*

Phytochemical screening of the seeds of *E. superbum,* revealed the presence of alkaloids, phenols, glycosides, steroids and sugars (Vasundharan *et al.,* 2013), while the pseudostem showed the presence of alkaloids, sterols, tannins, flavonoids, proteins and sugars (Sethiya *et al.,* 2015).

A compound whose structure was established as 4- (4-hydroxy-3-methyl-hex-5-enyl)-chroman-2,7-diol **(XVIII)** was isolated and characterized from the seeds of *Ensete Superbum* (Kachroo and Agrawal, 2009).
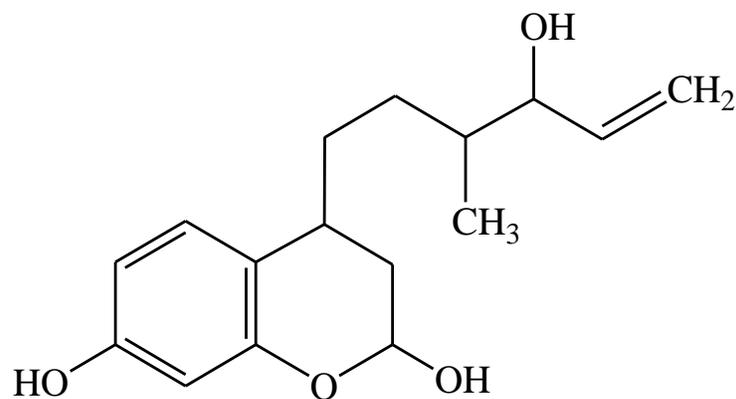
(XVIII)

Also, the GC-MS analysis of the seed extract of *E. superbum* revealed the presence of n-hexadecanoic acid, 9-eicosyne, 3-decanynoic acid, 1-tetradecyne, 7-methyl-Z-tetradecen-1-ol acetate **(XIX)**, 1-hexadecyne, eugenol **(XX)**, Z- (13,14-epoxy)tetradec-11-en-1-ol acetate, octadecanoic acid **(XXI),** tridecanedial and cis-13-eicosenoic acid (Kumar *et al.,* 2018). HPLC analysis of the seed extract revealed that it mainly contains flavonoids such as; gallic acid, caffeic acid and rutin, while FTIR analysis showed the presence of alkynes, alkanes, amines, aromatic amines, alkyl halides, alkenes, carboxylic acids and aromatic compounds (Kumar *et al.,* 2018).
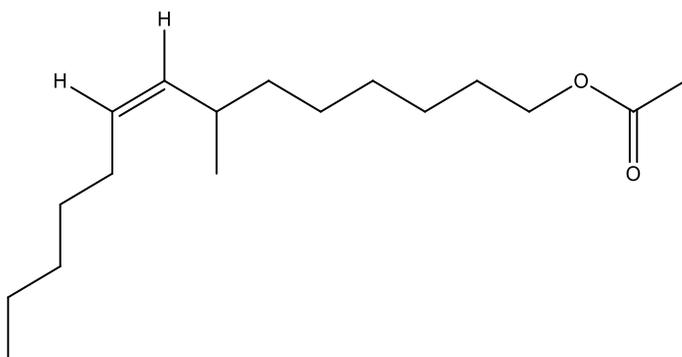
(XIX)
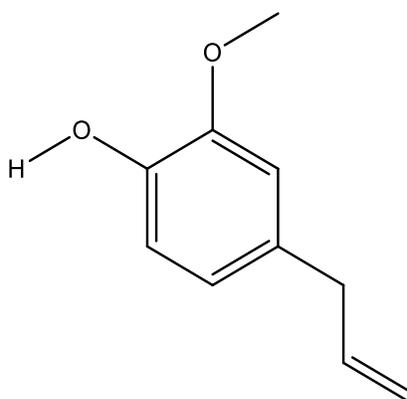
(XX)
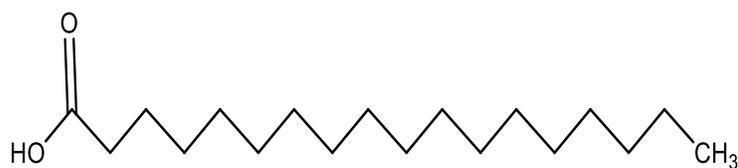
(XXI)

2.4 Biological Assay

2.4.1 *Ensete gilletii*

The proximate analysis carried out on the seeds of *Ensete gilletii* fruits, revealed that the seeds are highly rich in carbohydrates and protein which makes it suitable for dieting. Antimicrobial screening of the seed extracts revealed that the ethyl acetate and ethanol extracts were potent against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Candida glabrata, Candida krusen* and *C*andida *albicans*, while the n-hexane extract did not show any activity against all the test organisms used. All the extracts were reported to have antioxidant activity (Afolayan *et al.,* 2014). Studies on the seed extracts has proven that seeds of *Ensete gilletii* could be used as

an inhibitor of DPPH radical scavenging effect. There are limited studies on the biological activities of *E. gilletii* but there are documentations on other species.

2.4.2 *Ensete ventricosum*

Potential probiotic bacteria (*Lactobacilli sp.*) was isolated from traditionally fermented *Ensete ventricosum*. According to the report, the isolate was biologically active and conformed to the required criteria for a probiotic. The plant extracts have been reported to be potent against viral, bacterial, fungal and nematodal diseases of humans (Demissie and Abera, 2015). The pseudostem and seeds of *E. ventricosum* were analysed for their anti-viral and anti-fertility efficacy (Vasundharan *et al.,* 2013; Sethiya *et al.,* 2016). Chromatin derivative isolated from seeds of *E.ventricosum* can be used as a marker for elaborate antifertility studies (Sethiya *et al.,* 2016).

2.4.3 *Ensete superbum*

Documented reports on *Ensete superbum*, stated that the specie could be used for genetic improvement and conservation of *Ensete* (Diro and Staden, 2005). An isolated fraction from the seeds of *Ensete superbum* revealed potent anti-implantation activity (Kachroo and Agrawal, 2009). Pharmacognostic studies on the pseudostem revealed the presence of epidermis, hypodermis vascular bundles and phloem fibers. It also showed significant antioxidant activity (Sethiya *et al.,* 2015), this was reported to be as a result of the phenolic and flavonoids constituent present in the sample (Chang *et al.,* 2002). 4- (4-hydroxy-3-methyl-hex-5-enyl)-chroman-2,7-diol (chromatin derivative) isolated from seeds of *Ensete superbum* was validated by HPLC and HPTLC as a marker compound for elaborate antifertility studies (Kachroo and Agrawal, 2010; 2011). The

compound finds use in the production of medicine, cosmetics and as food additive to reduce cholesterol (Azene *et al.,* 2016).

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1 Materials

3.1.1 Instruments/Reagents/Solvents

High grade organic solvents, pre-coated TLC plates, different meshes of silica gel, filter paper of different sizes and sampling bottles. All the reagents used for this present study were of analytical grade.

Soxhlet extractor, glass columns with flash, rotary evaporator, water bath, weighing balance.

Gas chromatography-Mass spectrometry (GC-MS): Shimadzu QP-2010, GC with QP-2010 Mass selective Detector.

Ultra violet spectrophotometer model: 752 and 1800

Nuclear Magnetic Resonance (NMR)

3.1.2 Analytical techniques

Thin Layer Chromatography (TLC): pre-coated plates (0.25 mm thickness)

High Performance Thin Layer Chromatography (HPTLC): pre-coated plates

Preparative Thin Layer Chromatography (PTLC): pre-coated plates (1.00 mm thickness)

Column Chromatography (CC): 45 cm^3 by 3 cm^3 and 25 cm^3 by 2 cm^3

3.2 Extraction Procedures

3.2.1 Collection, identification and drying of plant material

Fresh roots of *Ensete gilletii* were collected from a farmland in Sarkin Pawa of Munya Local Government area of Niger State, Nigeria, during the wet season (July). The plant sample was authenticated at National Institute of Pharmaceutical Research Development (NIPRD), Idu, Abuja (Voucher number: NIPRD/H/6991). Fresh roots of *E. gilletii* were thoroughly washed and air dried in an open space for several weeks. The dried sample was pulverized to obtain fine particle size.

3.2.2 Extraction

Pulverized air dried plant sample (500 g) was extracted exhaustively with petroleum ether (40-60°C) using a soxhlet apparatus for a week, until colourless extractant was obtained. The resulting mixture was concentrated using a rotary evaporator and finally dried over a water bath. Extract was weighed and coded "PEG".

Defatted marc was air dried and further extracted with chloroform following the same procedure as above. The resulting extract was weighed and coded "CEG". The same process was repeated with methanol and the extract obtained was weighed and coded "MEG".

3.3 Qualitative Screening of Crude Plant Extracts

The three crude extracts (petroleum ether, PEG, chloroform, CEG and methanol, MEG extracts), obtained from section 3.2.2 were screened for the presence/absence of various phytochemicals using standard methods of analysis (Harbone, 1973; Trease and Evans, 1989; Sofowora, 1993; AOAC, 2005; Oloyede, 2005).

3.3.1 Test for alkaloids

Each extract (0.5 g) was dissolved individually in dil HCl, heated over a water-bath and filtered. Each filtrate was subjected to the following tests:

Mayer's test

Each filtrate (1 cm^3) was treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test

Each filtrate (1 cm^3) was treated with Wagner's reagent (iodine in potassium iodide). Formation of a reddish-brown precipitate indicates the presence of alkaloids.

3.3.2 Test for flavonoids

Each extract (0.5 g) was dissolved in distilled water and filtered. The filtrate of each was subjected to the following tests:

Alkaline test

Each filtrate was treated with few drops of NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

H_2SO_4 test

Dilute ammonia (5 cm^3) was first added to each filtrate, followed by conc H_2SO_4 (1 cm^3). A yellow colouration that disappears on standing indicates the presence of flavonoids.

3.3.3 Test for phenols

Ferric chloride test

Each extract (0. 5 g) was dissolved in distilled water and filtered. Each filtrate was treated with $FeCl_3$ 3 drops solution. Formation of bluish black colour indicates the presence of phenols.

3.3.4 Test for tannins

Gelatin test

Each extract (0.5 g) was dissolved in distilled water and filtered. To each filtrate, 1% gelatin solution containing NaCl was added. Formation of white precipitate indicates the presence of tannins.

Braymer's test

Each extract (0.5 g) was dissolved in distilled water 2 cm^3, boiled and filtered. In each test tube containing the filtrate, three drops of 5% ferric chloride solution was added. The formation of greenish-blue or a brownish-green color indicates the presence of tannins.

3.3.5 Test for saponins

Foam/Froth test

Each extract (0.5 g) was shaken with distilled water 2 cm^3. Persistence of foam for ten minutes indicates the presence of saponins.

3.3.6 Test for terpenoids

Salkowski's test

Each extract (0.5 g) was dissolved in chloroform 2 cm^3 and filtered. Conc H$_2$SO$_4$ (3 cm^3) was carefully added to the filtrate to form a layer. A reddish-brown colouration at the interphase indicates the presence of terpenoids.

3.3.7 Test for phytosterols

Liebermann Burchard's test

Each extract (0.5 g) was dissolved in $CHCl_3$ and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 was added carefully, formation of brown ring at the junction indicates the presence of phytosterols.

3.3.8 Test for cardiac glycosides

Keller-Killiani's test

Each extract (0.5 g) was mixed with 2 cm^3 of glacial acetic acid containing 2 drops of 2% $FeCl_3$ solution. 2 cm^3 of conc. H_2SO_4 was carefully added from the side of each test tube. A reddish-brown ring at the interphase indicates the presence of cardiac glycosides.

3.4 Quantitative Screening of Plant Material

Quantitative tests to determine amount of various classes of phytoconstituents in the air-dried pulverized plant material was carried out using standard procedures (Harborne, 1973; Sofowora, 1993; Chang, 2002; Oloyede, 2005).

3.4.1 Determination of saponins (Oloyede, 2005)

Plant sample (0.5 g) was weighed into a test tube and HCl (20 cm^3) was added, the mixture was then boiled HCl for 4 h. After cooling, the mixture was filtered and 50 cm^3 of petroleum ether was added to the ethereal layer (filtrate) and evaporated to dryness and then, acetone in ethanol 5 cm^3 was added. The mixture 0.4 cm^3 was taken in a test tube and ferrous sulphate reagent 6 cm^3 and conc H_2SO_4 2 cm^3 added. It was thoroughly mixed and the absorbance of the resultant solution was taken at 490 nm after 10 mins.

3.4.2 Determination of tannins (Sofowora, 1993)

Plant sample (0.5 g) in 50% methanol 20 cm^3; was covered with para film and placed over a water bath at 77-80$^\text{O}$C for 1 h. The mixture was shaken thoroughly and was filtered using a double layered Whatman No.41 filter paper into a 100 cm^3 volumetric flask. 20 cm^3 water, 2.5 cm^3 Folin-Denis reagent and 10 cm^3 of Na_2CO_3 were added to the flask and mixed properly. The mixture was made up to mark with water, mixed well and allowed to stand for 20 min for the appearance of a bluish-green colour. The absorbance of the tannic acid solution was read at a wavelength of 760 nm.

3.4.3 Estimation of total phenols (Sofowora, 1993)

Plant sample (0.5 g) with dilute Folins-phenol reagent in water (1:1) was heated for 15 mins and filtered. 2.5 cm^3 of 20% Na_2CO_3 was added to the filtrate for the extraction of the phenolic components. 2 cm^3 of NH_4OH solution was further added. The mixture was left to react for 30 min for colour development. The absorbance of the resultant solution was read at 725 nm.

3.4.4 Estimation of alkaloids (Harbone, 1973)

 The plant sample (0.5 g) was weighed into a beaker 250 cm^3 and 10% acetic acid in ethanol 200 cm^3 was added, mixture was covered and allowed to stand for 4 hrs. The mixture was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume; conc NH_4OH was added drop-wisely to the filtrate until precipitation was complete. The solution was allowed to settle, precipitate collected, washed with dilute NH_4OH, filtered and weighed. The percentage of total alkaloids content was calculated as:

Percentage of total alkaloids (%) = weight of precipitate $\times$ 100/ weight of sample taken.

3.4.5 Estimation of total flavonoids (Chang, 2002; Krishnaiah *et al.,* 2009)

Chang (2002)/(AlCl$_3$ colorimetric method): Plant sample (0.5 g) was mixed with methanol 1.5 cm^3, 10% AlCl$_3$ 0.1 cm^3, 1M sodium acetate 0.1 cm^3 and distilled water 2.8 cm^3 and kept at room temperature for 30 mins. The absorbance of the reaction mixtures was measured at 415 nm. The calibration curve was prepared by using quercetin solution at concentrations of 12.5 to 100 g/cm^3 in methanol and the flavonoid content was determined from the calibration curve.

Krishnaiah *et al.* (2009) method: dried plant sample (0.5 g) was repeatedly extracted with 80% aqueous methanol 100 cm^3 at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250 cm^3 beaker. The filtrate was transferred onto a water bath and allowed to evaporate to dryness. To the methanol extract 0.1 g, distilled water 5 cm^3 and sodium nitrite 5 cm^3 were added and allowed to stand for colour change. The absorbance was taken at 725 nm.

3.5 Isolation and Characterisation of Compound from Crude Petroleum ether Extract (PEG)

3.5.1 Thin layer chromatography (TLC) of PEG

Pre-coated TLC plate was spotted with a very dilute solution of PEG dissolved in hexane, plate was allowed to dry and developed using CHCl$_3$ (100%) as the mobile phase. Number of spots on developed chromatogram was visualised using (i) sunlight (ii) iodine vapor (iii) (10% H$_2$SO$_4$) as a chromogenic spray reagent and heated in an oven to 105°C. Retention factor (R$_f$) for each spot was calculated as follows:

R$_f$ = distance travelled by spot of PEG

distance travelled by solvent

3.5.2 GC-MS analysis of PEG

The petroleum ether crude extract (PEG, 0.1 g) was dissolved in hexane 5 cm^3 and subjected to gas chromatography-mass spectrometer (GC-MS) model; Shimadzu QP-2010 GC with QP-2010 Mass selective Detector (MSD, operated in the EI mode (electron energy =70 eV), scan range of 45-700 amu, and scan rate of 3.99 scans/sec], and Shimadzu GC-MS solutions data system. The gas chromatography column was optima-5 ms fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 μm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for gas chromatography oven temperature was 60-180°C at a rate of 12°C/min, then held at 180°C for 0 min, followed by 180-280°C at a rate of 12°C/min then again held at 280°C for 2 min. The injection port temperature was 250°C while detector temperature was 280°C. 1.0 μL of diluted sample (500 μg/cm^3 in solvent, w/v) was injected using auto-sampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current.

3.5.3 Fractionation of PEG

The petroleum ether crude extract (PEG) was fractionated using flash column chromatography. PEG 5 g was mixed with little quantity of silica gel, dried and powdered. Sample was introduced into a glass column packed with 150 g of silica gel (mesh 60-120) by the slurry method using petroleum ether (100%) as the mobile phase. Sequentially, a mixture of varying proportions of

increasing polarity of Pet. ether: $CHCl_3$ and $CHCl_3$: EtOAc (100:0 to 0:100) was used as the eluting solvents giving a total of 240 fractions as eluents (20 cm^3 each). The eluted fractions were monitored by thin layer chromatography using ($CHCl_3$ 100%, PE: EtOAc: 4:1) as mobile phases and similar fractions pooled to yield seven major fractions coded "PEG1 to PEG7", based on their TLC profiles.

3.5.4 Re-fractionation of Fraction PEG3

Fraction PEG3 (0.9 g) was re-fractionated using a small sized flash column. Same procedure as 3.5.3 was adopted and a total of 50 fractions were collected. Fractions were monitored on TLC ($CHCl_3$:100%, Hexane: EtOAc: 5:1) and pooled into 3 major sub-fractions coded "PEG3a, PEG3b and PEG3c".

3.5.4.1 Purification of PEG3c

Further purification of sub-fraction PEG3c was carried out using preparative thin layer chromatography (PTLC). Pre-coated PTLC plate was activated in an oven (120°C) and allowed to cool. A pencil was used to gently mark a line (1 inch) as the origin. Using a capillary tube, fairly dilute mixture of sub-fraction PEG3c dissolved in CH_2Cl_2 was carefully deposited horizontally on the line, repeating the process to increase concentration. The plate was allowed to dry and developed in CH_2Cl_2 (100%). The upper band (higher R_f value) of developed PTLC plate having distinct separation and concentration was scraped using a spatula. The silica gel containing band was dispersed in acetone and allowed to stand. The mixture was filtered and the resulting filtrate concentrated in vacuo to afford a compound coded PEG3c1. TLC of the compound on high performance thin layer chromatography (HPTLC) plate using hexane: EtOAc (5:1) as mobile

phase revealed a single spotted compound on spraying the dried chromatogram with 10% H_2SO_4 and heated in an oven to 105^oC.

3.5.4.2 Characterisation of compound PEG3c1

The isolated compound (PEG3c1) was characterised using:

(i)   Physical tests: appearance, colour, weight, solubility, TLC.

(ii)   Chemical test for class of compound using standard methods.

       The isolated compound was dissolved in dilute ammonia and shaken. Mixture was allowed to stand for 5 mins. The appearance of red or yellow colour indicates free anthracenes.

(iii) Spectral analysis: ^1HNMR, $^{13}CNMR$.

3.6 Purification of Column Fraction PEG7

Column fraction PEG7 that was obtained from fractionation (section 3.5.3) was further purified by washing severally with petroleum ether. The PEG7 was dissolved in petroleum ether using a separating funnel; it was allowed to stand for 3 h before decanting. After decanting, the petroleum ether soluble filtrate was allowed to gel in an open space and coded "PEG7a". The process was repeated with PEG7a to obtain a filtrate coded "PEG7b" which was subjected to same process and the resulting filtrate was coded "PEG7c".

3.6.1 TLC of PEG7

Same process for TLC as sub-section 3.5.1 was adopted. Filtrates were subjected to TLC using mobile phase solvent systems;

(i)   CHCl₃:100%

(ii)   PE: EtOAc: 4:1

(iii)   Hexane: EtOAc: 5:1

(iv)   CH₂Cl₂:100% were used to monitor the separation.

3.7 GC-MS of Chloroform Crude Extract of *Ensete gilletii* (CEG)

The chloroform crude extract (CEG) was analyzed for the presence and quantity of its phytoconstituents using gas chromatography-mass spectrometer (GC-MS) as outlined in sub-section 3.5.2.

3.8 Antibacterial Susceptibility Test

3.8.1 Collection/sterilisation of test organisms

Pure isolates of organisms for this study were obtained from Microbiology Department, Federal University of Technology, Minna, Niger state.

3.7.2 Reconstitution of plant extracts

The stock solution of each extract was prepared by dissolving 0.4 g each of the extract in 5 cm^3 of dimethyl sulfoxide (DMSO) solvent to obtain 80 mg/cm^3. All were dispensed in sterile McCartney bottles.

3.7.3 Standardisation of inoculum

Isolates were inoculated into 20 cm^3 of nutrient broth and incubated for 24 h at 37°C.

0.2 cm^3 was taken from 24 h culture and inoculated into another 20 cm^3 nutrient broth and incubated for 2 h which is equivalent to 10^6cfu/cm^3 McFarland's standard.

3.7.4 Evaluation of antibacterial activity

Agar well diffusion method as described by NCCS (2003) was adopted for this study. Muller Hinton agar was prepared according to manufacturer's guide and autoclaved at 121°C for 15 min. 20 cm^3 molten agar was dispensed in Petri dishes aseptically and allowed to gel. 5 mm cork borer was used to bore wells on the agar. The standardized inoculum was swabbed on the surface of the agar. The wells were filled with reconstituted extracts and allowed to stand for one hour. For diffusion to take place, the plates were incubated at 37°C for 24 h. The procedure was repeated using a standard drug (ampicillin) as a positive reference. The zones of inhibition were measured and recorded in millimeters.

3.7.5 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined according to the National Committee for Clinical Standard (2003). 2 cm^3 of nutrient broth was dispensed into pre-sterilised test tubes and 2 cm^3 of each reconstituted extract was added and serial diluted. To a test tube containing 2 cm^3 of nutrient broth, ampicillin was added. All the test tubes were incubated at 37°C for 24 h. The test tube with no visible growth of organism is referred to as MIC.

3.7.6 Determination of maximum bactericidal concentration (MBC)

National Committee for Clinical Standard method was adopted for the minimum bactericidal concentration study. From the MIC results, that is, the test tube that showed no visible growth or turbidity were plated out on a sterilized Muller Hinton agar and incubated at 37°C for 24 h. The least concentration with no visible growth was recorded as MBC.

CHAPTER FOUR

4.0   **RESULTS AND DISCUSSION**

4.1 RESULTS

4.1.1 % Extractable and physical appearance of crude extracts

The dried pulverized roots of *E.gilletii* (500 g) was subjected to exhaustive and successive extraction using three different solvents of increasing polarity. The physical appearances of each extract and their weight (in grams) is presented in Table 4.1.

Table 4.1: % Extractable and Physical Appearance of Crude Extracts

Extract	Code	Physical appearance	Weight (g)	% Extractable
Pet. Ether	PEG	oily green mass	32.4	6.5
Chloroform	CEG	reddish-brown mass	30.4	6.1
Methanol	MEG	dark brown gummy mass	42.5	8.5

4.1.2 Qualitative screening of *E. gilletii* root extracts

All three extracts obtained from the roots of *E. gilletii* were screened for the presence/absence of various phytoconstituents using standard methods as presented in Table 4.2.

Table 4.2: Qualitative Screening of *Ensete gilletii* Root Extracts

Phytoconstituents	Test	Observation	Inference: PEG	CEG	MEG
Alkaloids	Mayer's test	no precipitate	_	_	_

			PEG	CEG	MEG
	Wagner's test	no precipitate	–	–	–
Flavonoids	Alkaline test	yellow colour	_	++	++
	H_2SO_4 test	yellow colour	_	++	++
Glycosides	Keller-Killiani's test	reddish-brown ring	–	+	++
Phenols	$FeCl_3$ test	bluish-black colour	+++	+++	+++
Tannins	Gelatin test	white precipitate	_	_	++
	Braymer's test	brownish-green colour	–	+	++
Terpenoids	Salkowski's test	reddish-brown colour	+++	+++	+++
Saponins	Foam test	persistent foam	_	_	+
Phytosterols	Keller-Killiani's test	violet ring	+	+	+

Keys: - absent + present ++ moderately present +++ abundant

PEG: Petroleum ether extract

CEG: Chloroform extract

MEG: Methanol extract

4.1.3 Quantitative screening of *E. gilletii* roots

The quantitative screening of some phyto-constituents present in the dried pulverized *E. gilletii* roots was carried out in triplicates. Analysis of variance (ANOVA) was used to generate the mean value which is presented in Figure 4.1.
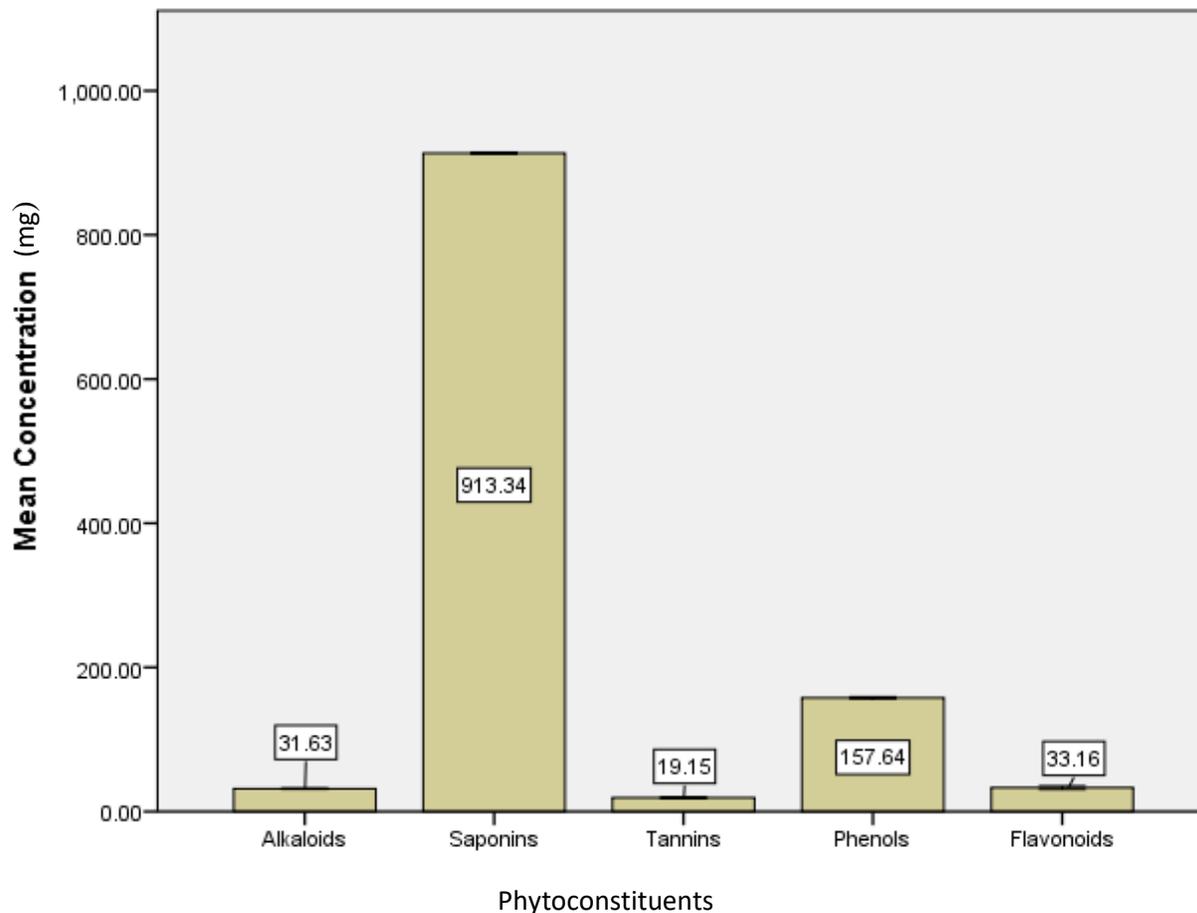
Figure 4.1: Quantitative Screening of *Ensete gilletii* Dried Root Sample (mg/100 g)

4.1.4 GC-MS analysis of crude petroleum ether extract of *E. gilletii* roots

GC-MS analysis of the petroleum ether crude extract (PEG) revealed the presence of eighty compounds which has been classified with their relative % abundance in the plant as shown in Table 4.3.

Table 4.3: Phytochemical Compounds in Pet.ether Extract of *Ensete gilletii* Roots Based on GCMS Analysis

Chemical Classes	Name of Compound	Molecular Formula	Retention Time	Area %
Terpenes/Terpenoids	Hemimellitene	C_9H_{12}	5.795	1.31
	Mesitylene	C_9H_{12}	5.350	1.76
	PsiCumene	C_9H_{12}	4.967	0.84
	Cumene, p-ethyl-	$C_{11}H_{16}$	8.438	0.67
	p-Cymene	$C_{10}H_{14}$	6.297	3.00
	p-Mentha-1,3,8 triene	$C_{10}H_{14}$	6.710	2.20
	o-Xylene, 3-ethyl-	$C_{10}H_{14}$	6.584	1.85
	Isodurene	$C_{10}H_{14}$	7.216	1.04
	p-Ethylstyrene	$C_{10}H_{12}$	7.493	1.09
	Dihydrocurcumene	$C_{15}H_{26}$	7.959	0.66
	Bicyclo[4.2.1] nona-2,4,7-triene,7-et	$C_{11}H_{14}$	8.789	1.47
	alpha-Methylnaphthalene	$C_{11}H_{10}$	9.607	2.50
	Naphthalene,6-ethyl-1,2,3-4-tetrahy	$C_{11}H_{10}$	9.825	1.24
	1,8-Dimethylnaphthalene	$C_{12}H_{12}$	11.129	0.67
	Squalene	$C_{30}H_{62}$	14.013	1.94
	Phytane	$C_{20}H_{42}$	16.703	1.67
	(17E)-17-Pentatriacontene	$C_{35}H_{70}$	17.331	0.81
	Prehnitol	$C_{10}H_{14}$	7.152	0.85
	m-Propyltoluene	$C_{10}H_{14}$	6.196	1.19
	3-(2-Methyl-propenyl)-1H-indene	$C_{13}H_{14}$	12.557	0.74
	2-Naphthalenol,1,2-dihydro-acetate	$C_{12}H_{12}O_2$	8.151	2.36
	Guajen	$C_{12}H_{12}$	11.183	0.64
	(+)-3-Carene, 2-(acetylmethyl)-	$C_{13}H_{20}O$	7.550	0.76
	1,6-Dimethylindan	$C_6H_{16}N_2$	8.308	1.50
	4,7-Dimethylindan	$C_{11}H_{14}$	8.999	1.30
	Decyl alpha-d-glucoside, 3-acetyl-	$C_{18}H_{22}O_8$	9.068	0.84
Fatty Acids and their Esters	Pelargone	$C_{17}H_{34}O$	15.626	0.77
	Palmitic acid	$C_{16}H_{32}O_2$	16.391	1.30
	Malonic acid, 3-methylbutyl pentade	$C_{14}H_{26}O_s$	10.23	1.30
	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	16.068	0.66
	Oxalic acid, 2-ethylhexyl hexyl ester	$C_{16}H_{30}O_4$	8.925	0.73
	Bacteriochlorophyll-c-stearyl	$C_{52}H_{72}MgN_4O_4^{2-}$	14.853	1.51
	Myristyl alcohol	$C_{14}H_{30}O$	17.887	2.00
	Benzene, (1,3,3 trimethylnonyl)-	$C_{10}H_{30}$	4.864	1.89
	Benzene,4-(2-butenyl)-1,2-dimethyl	$C_{12}H_{16}$	9.886	0.59
	Benzene, 1-methyl-2-(2-propenyl)-	$C_{10}H_{12}$	7.651	2.29
Carbonyl	Methyl-2-methoxy-4-pentenoate	$C_7H_{13}O_2$	4.039	0.80
	p-Cumic aldehyde	$C_{10}H_{12}O$	6.975	0.65
	Biacetyl	$C_4H_6O_2$	14.917	1.94

Table 4.3 Cont'd

Chemical Classes	Name of Compound	Molecular Formula	Retention Time	Area %

Phenolic/alcoholic compounds	Henicosan-1-ol	$C_{21}H_{44}O$	18.293	1.54
	3-Phenyl-2-butanol	$C_{10}H_{14}O$	3.534	0.68
	Cosbiol	$C_{30}H_{62}$	17.53	1.29
	Behenic alcohol	$C_{22}H_{46}O$	16.65	1.58
	3-Phenyl-4,5-dimethyl-2,1-oxaborol	$C_{10}H_{12}N_2O_3$	9.426	1.40
	Phenol 2,4-di-ter-butyl	$C_{14}H_{22}$	12.059	0.74
Hydrocarbons	Nonane	C_9H_{20}	3.916	0.64
	Nonane, 5-(2-methylpropyl)-	$C_{13}H_{28}$	6.432	0.90
	Nonane,3-methyl-5-propyl	$C_{13}H_{28}$	9.527	1.22
	Decane	$C_{10}H_{22}$	5.397	1.83
	Decane, 4-methyl	$C_{11}H_{24}$	5.734	0.73
	1-Isopropyl-3-methylcyclohexane	$C_{10}H_{20}$	5.175	0.66
	Dodecane, 2-cyclohexyl-	$C_{18}H_{36}$	5.908	0.53
	Dodecane,4-cyclohexyl-	$C_{14}H_{28}$	4.402	0.72
	Decane 1-iodo-	$C_{12}H_{25}I$	19.830	0.93
	Eicosane	$C_{20}H_{42}$	12.941	1.89
	Undecane	$C_{11}H_{24}$	6.860	2.05
	n-Tridecane	$C_{14}H_{30}$	8.240	1.16
	Undecane, 2,8-dimethyl-	$C_{13}H_{28}$	7.838	2.12
	alpha- Tetradecane	$C_{14}H_{28}$	10.641	0.56
	n-Tetradecane	$C_{14}H_{30}$	10.734	1.21
	n-Heptadecane	$C_{17}H_{35}$	15.831	1.65
	Phytane	$C_{20}H_{42}$	16.703	1.67
	Henicosane	$C_{21}H_{44}$	13.955	2.02
	Tetracosane	$C_{24}H_{50}$	15.009	1.46
	5-Butylnonane	$C_{13}H_{28}$	15.300	1.05
	9-Methylnonadecane	$C_{20}H_{42}$	19.561	0.58
	Tetrapentacontane	$C_{54}H_{110}$	19.096	0.96
	Hexatriacontane	$C_{36}H_{74}$	18.334	1.15
	Pentadecanal	$C_{15}H_{30}$	7.095	0.60
	n-Pentadecyclohexane	$C_{21}H_{42}$	11.337	0.82
	2,3,7-Trimethyldecane	$C_{13}H_{28}$	11.445	1.07
	n-Cetane	$C_{16}H_{34}$	11.869	1.93
	Hexahydroaplotaxene	$C_{17}H_{34}$	12.863	1.30
	Norphytane	$C_{19}H_{40}$	13.438	0.78
	5-Propyltridecane	$C_{16}H_{34}$	14.375	1.05
	Dodecylcyclohexane	$C_{18}H_{36}$	14.561	0.85
	2-Methyldodecane	$C_{13}H_{28}$	15.626	0.93

4.1.5 Fractionation of crude petroleum ether extract of *E. gilletii* roots

Fractionation of petroleum ether extract (PEG) using column chromatography yielded seven major fractions. The mobile phases for elution, physical appearance, weight, % extractable of the sub-fractions are presented in Table 4.4.

Table 4.4: Description of Pooled Major Column Fractions from Fractionation of Crude Petroleum Ether Extract of *Ensete gilletii* (PEG)

Pooled Sub-fraction	Mobile phase	Code	Physical Appearance	Weight (g)	%Extractable
5-40	PE: CHCl$_3$ (9 :1)	PEG1	greenish-brown oily mass	1.3	26
41-64	PE : CHCl$_3$ (7 :1)	PEG2	reddish-brown gummy mass	0.4	8
65-92	PE : CHCl$_3$ (6 : 4)	PEG3	yellowish-brown gummy mass	0.9	18
93-104	PE : CHCl$_3$ (3 :7)	PEG4	brown gummy mass	0.2	4
105-158	PE : CHCl$_3$ (1 :1)	PEG5	dark brown gummy mass	0.4	8
159-170	CHCl$_3$ (100%)	PEG6	dark brown mass	0.2	4
171-240	CHCl$_3$:EtOAc (9 :1)	PEG7	black mass	1.4	28

Key: PE= petroleum ether

4.1.6 TLC of pooled fractions PEG1 – PEG7

The seven major fractions pooled from fractionation of crude petroleum ether extract, PEG were analyzed using TLC. The number of spots, their R_f values and colours observed with sunlight, iodine vapour (I_2) and Liebermann Burchard's reagents are presented in Table 4.5.

Table 4.5: TLC Profile of Pooled Major Fractions from Column Fractionation of Petroleum Ether Extract (PEG)

Fraction	Mobile phase	Number of spots on TLC	R_f value	Colours observed with:		
				Sunlight	I_2	LBR
PEG1	CHCl$_3$ (100%)	6	0.9	dark brown	brown	-
			0.11	green	dark green	-
	PE: CHCl$_3$ (3 :1)	6	0.15	brown	brown	-
			0.18	green	dark green	-
			0.20	brown	brown	-
			0.22	brown	brown	-
PEG2	PE: CHCl$_3$ (1:1)	4	0.1	Brown	brown	-
			0.04	brown	brown	-
	CHCl$_3$ (100%)		0.18	brown	brown	-
			0.24	brown	brown	-
PEG3	CHCl$_3$ (100%)	2	0.56	Brown	brown	Darkpurple
			0.58	purplish brown	purplishbrown	Darkpurple
PEG4	CHCl$_3$ (100%)	3	0.22	Brown	brown	-
			0.3	brown	brown	-
			0.4	brown	brown	-
PEG5	CHCl$_3$ (100%)	3	0.1	Brown	brown	-
			0.3	brown	brown	-
			0.5	brown	brown	-
PEG6	CHCl$_3$ (100%)	3	0.04	Brown	brown	-
			0.08	brown	brown	-
			0.14	brown	brown	-
PEG7	PE:EtOAc (3:1)	2	0.58	Brown	brown	Darkpurple
			0.60	Brown	brown	Darkpurple

Keys: LBR= Liebermann Burchard's reagent, PE= petroleum ether, I_2 = iodine vapour,   - = not sprayed.

4.1.7 TLC summary of pooled fractions PEG1 – PEG7

The TLC profiles of the seven major fractions (PEG1 to PEG7) from column fractionation of crude petroleum ether extract of *E. gilletii* (PEG), is summarized in Table 4.6. Developed TLC chromatograms are presented in Plates 4.1 – 4.3.

Table 4.6: TLC Profile of 7 Major Column Fractions

Sub-fractions	Code	Solvent system	Number of spots on TLC	Remarks
5-40	PEG1	$CHCl_3$ (100%) PE: $CHCl_3$ (3 :1)	6	several tiny spots with close Rf values
41-64	PEG2	PE: $CHCl_3$ (1:1) $CHCl_3$ (100%)	4	several spots with close Rf values
65-92	PEG3	$CHCl_3$ (100%)	2	well resolved spots
93-104	PEG4	$CHCl_3$ (100%)	3	well resolved spots
105-158	PEG5	$CHCl_3$ (100%)	3	well resolved spots
159-170	PEG6	$CHCl_3$ (100%)	3	several spots with close Rf values
170-240	PEG7	$CHCl_3$ (100%) PE:EtOAc (3:1)	2	well resolved spots

Plate I: TLC Chromatograms of PEG1, PEG2, PEG3 & PEG4 in I₂ vapour

Solvent system: PE: CHCl$_3$ (3:1), CHCl$_3$ (100%)

Plate II: TLC Chromatograms of PEG5, PEG6 & PEG7 in I₂ vapour

**Solvent system:** CHCl$_3$ (100%), PE: EtOAc (3:1)
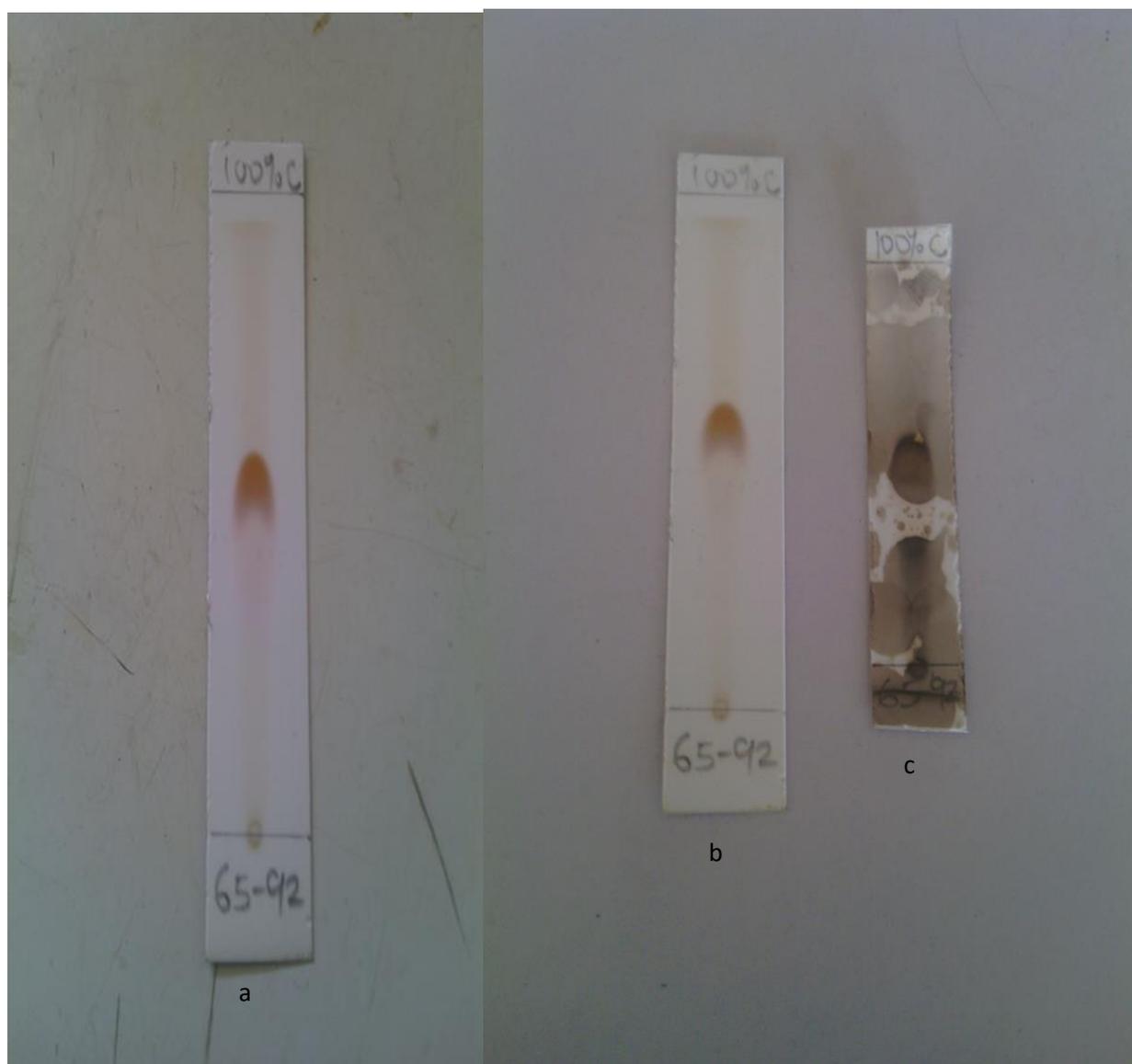
Plate III: TLC Chromatograms of fraction PEG3 in (a) sunlight (b) I₂ vapour

(c) Liebermann Burchard's reagent

Solvent system: $CHCl_3$ (100%)

R_f value: 0.56, 0.58

4.1.9 Re-fractionation of Fraction PEG3

Re-fractionation of Fraction PEG3 using column chromatography yielded three major sub-fractions. The mobile phases, physical appearance, weight and % extractable are presented in Table 4.7.

Table 4.7: Further Re-fractionation of PEG3

Pooled subfractions	Solvent System	Code	Physical appearance	Weight (g)	% Yield
6-14	PE : EtOAc (9:1)	PEG3a	brown gummy mass	0.2	22
15-31	PE : EtOAc (4:1)	PEG3b	reddish-brown gummy mass	0.28	31
32-50	PE : EtOAc (1:1)	PEG3c	yellowish-brown viscous mass	0.4	44

Key: PE= petroleum ether

4.1.10 TLC of pooled sub-fractions from re-fractionation of PEG3

Three major sub-fractions pooled from re-fractionation of PEG3 were analyzed using TLC. The number of spots, their R_f values and colours observed with sunlight, iodine vapour (I_2) and H_2SO_4 are presented in Table 4.8. Developed TLC chromatograms for PEG3c are presented in Plates 4.4 – 4.5.

Table 4.8: TLC Profile of 3 Major Sub-fractions of Fraction PEG3

Sub-fractions	Mobile phase	Number of TLC spots	R_f value	Colours observed with: Sunlight	I_2	10% H_2SO_4
PEG3a	$CHCl_3$ (100%)	2	0.5	Brown	burnt orange	-
			0.54	Brown		
PEG3b	$CHCl_3$ (100%)	2	0.45	Brown	brown	-
			0.5	brown	brown	-
	PE: EtOAc (4:1)	2	0.5	light yellow	brown	-
			0.6	light yellow	brown	-
PEG3c	PE : EtOAc (4 :1)	2	0.6	purple ring	dark yellow	yellow
	PE : $CHCl_3$ (3:1)	2	0.58	light yellow		
	$CHCl_3$ (100%)	2	0.54	purple ring	dark yellow	yellow
	CH_2Cl_2 (100%)	2	0.6	yellow	dark yellow	
			0.7	purplish yellow		yellow
	Hex : EtOAc (5:1)	2	0.45		dark yellow	
			0.7	light yellow	dark yellow	
					dark yellow	
					darkyellow	

Keys: PE= petroleum ether, Hex= hexane, I_2= iodine vapour, **-** = not sprayed

Plate IV: TLC chromatograms of sub-fraction (PEG3c)

Solvent system:

a= PE: CHCl$_3$ (3:1)

b= PE : EtOAc (4:1)

c= CHCl$_3$ (100%)
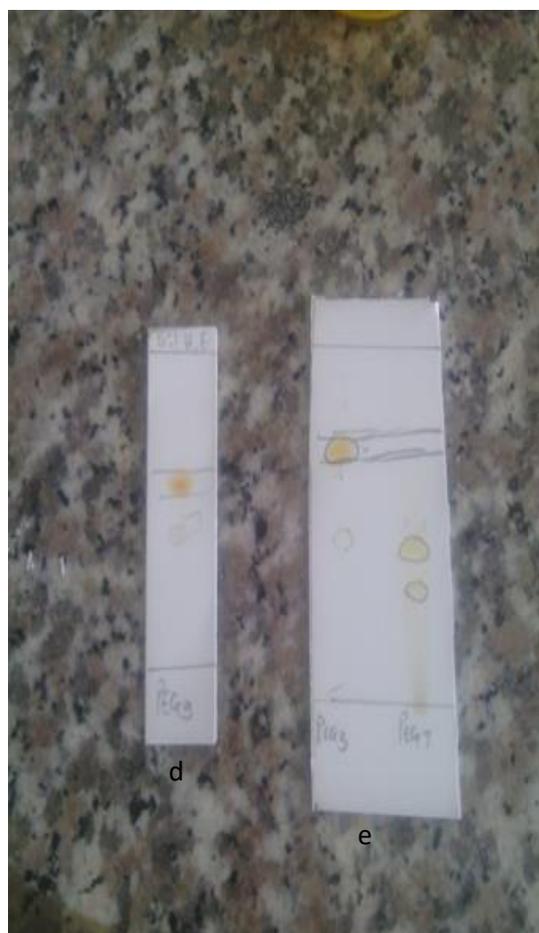
Plate V: TLC chromatograms of sub-fraction (PEG3c)

Solvent system:

d= Hex: EtOAc(5:1)

e= CH$_2$Cl$_2$ (100%) sprayed with 10%H$_2$SO$_4$

4.1.11 Purification of sub-fraction PEG3c

Sub-fraction PEG3c was further purified using preparative thin layer chromatography (PTLC), CH_2Cl_2 (100%) was used as the mobile phase. Upper layer of the PTLC plate was scraped, washed in acetone and filtered. The resulting filtrate was dried in vacuo and coded PEG3c1. PEG3c1 was spotted on high performance thin layer chromatography (HPTLC) plate.

TLC of PEG3c1 solvent system: Hexane: EtOAC (5:1)

4.1.11.1 physical tests of compound PEG3c1

 Developed chromatogram revealed spot observed with;

  (i)   Sunlight: yellow colour

  (ii) I_2 vapour: yellow colour

  (iii)   On spraying with 10% H_2SO_4: yellow colour

Appearance of the compound: viscous oily mass

Colour of the compound: yellow

Weight: 10 mg

Solubility: hexane (very soluble), MeOH (partial soluble), water (insoluble)

Plate 4.6 shows developed chromatogram.

Plate VI : TLC chromatogram of isolated compound (PEG3c1)

Solvent system: Hexane: EtOAc (5:1) sprayed with 10% H_2SO_4

R_f value: 0.52

4.1.12 Spectral characterisation of isolated compound

The structural elucidation of PEG3c1 was done using physical parameters; appearance, colour, weight, solubility and TLC as shown in 4.1.11 also, chemical test and Instrumental techniques; ^{1}HNMR spectrum, ^{13}CNMR spectrum.

Chemical Test: the isolated compound (PEG3c1) was positive to anthracene test with the appearance of a yellow colouration, which indicates the presence of free anthracene.

14.1.12.1 ^{13}C NMR of compound PEG3c1

The ^{13}C NMR of compound PEG3c1 is summarized in Table 4.10 as shown in Figure 4.2.

Table 4.10: Summary of ^{13}C-NMR spectral data of compound PEG3c1

Position	^{13}C (ppm)	Assignment	Literature values (ppm)
C-1	23.7	-CH$_2$	31.9
C-2	23.0	-CH$_2$	29.3
C-3	34.4	-CH$_2$	38.4
C-4	68.2	H-C-OH	69.8
C-5	29.1	-CH$_2$	36.2
C-6	60.1	H-C-OH	67.6
C-7	28.9	-CH$_2$	36.2
C-8	65.0	H-C-OH	69.8
C-9	30.6	-CH$_2$	37.8
C-10	22.7	-CH$_2$	27.3
C-11	19.7	-CH$_2$	23.0
C-12	11.0	-CH$_3$	14.1
C-13	131.1	Quaternary carbon –C=C- (Ring A)	135.3
C-14	128.8	-C=C- in (Ring B)	126.7
C-15	127.8	-C=C- in (Ring B)	125.9
C-16	130.7	Quaternary carbon of –C=C- (Ring B)	130.1
C-17	31.9	-C-C- in (Ring B)	38.1
C-18	135.6	Quaternary carbon of –C=C- (Ring A)	139.9
C-19	37.4	-CH$_2$-	42.4
C-20	29.7	-CH$_2$- (Ring C)	36.9
C-21	132.3	Quaternary carbon of –C=C- (Ring D)	135.5

Table 4.10 Cont'd

Position	¹³C (ppm)	Assignment	Literature values (ppm)
C-22	129.8	-C=C- (Ring C)	128.1
C-23	130.9	-C=C- (Ring D)	132.1
C-24	37.1	–C-C- (Ring D)	38.7
C-25	38.7	-C-C in cyclohexane	47.2
C-26	27.1	R-C-	33.3
C-27	30.0	–C- in cyclohexane (Ring E)	37.5
C-28	25.0	-C-C-	32.8
C-29	28.0	-CH$_2$ (Ring E)	34.0
C-30	26.7	-CH$_2$- (Ring E)	33.0
C-31	112.8	-C=C- (Ring A)	122.9
C-32	127.0	-C=C- (Ring A)	124.5
C-33	127.6	-C=C- (Ring A)	125.8
C-34	128.3	-C=C- (Ring A)	126.2
C-35	13.7	-CH$_3$	19.6
C-36	29.4	-CH-	36.6
C-37	32.7	-CH$_2$-	38.2
C-38	14.1	-CH$_2$-	20.5
C-39	0.03	H$_3$C-	14.4
C-40	19.2	-CH$_3$	21.0

Source: Laurie S. Starkey, Organic Chemistry Lab CHM 318L ¹³C NMR Chemical Shifts
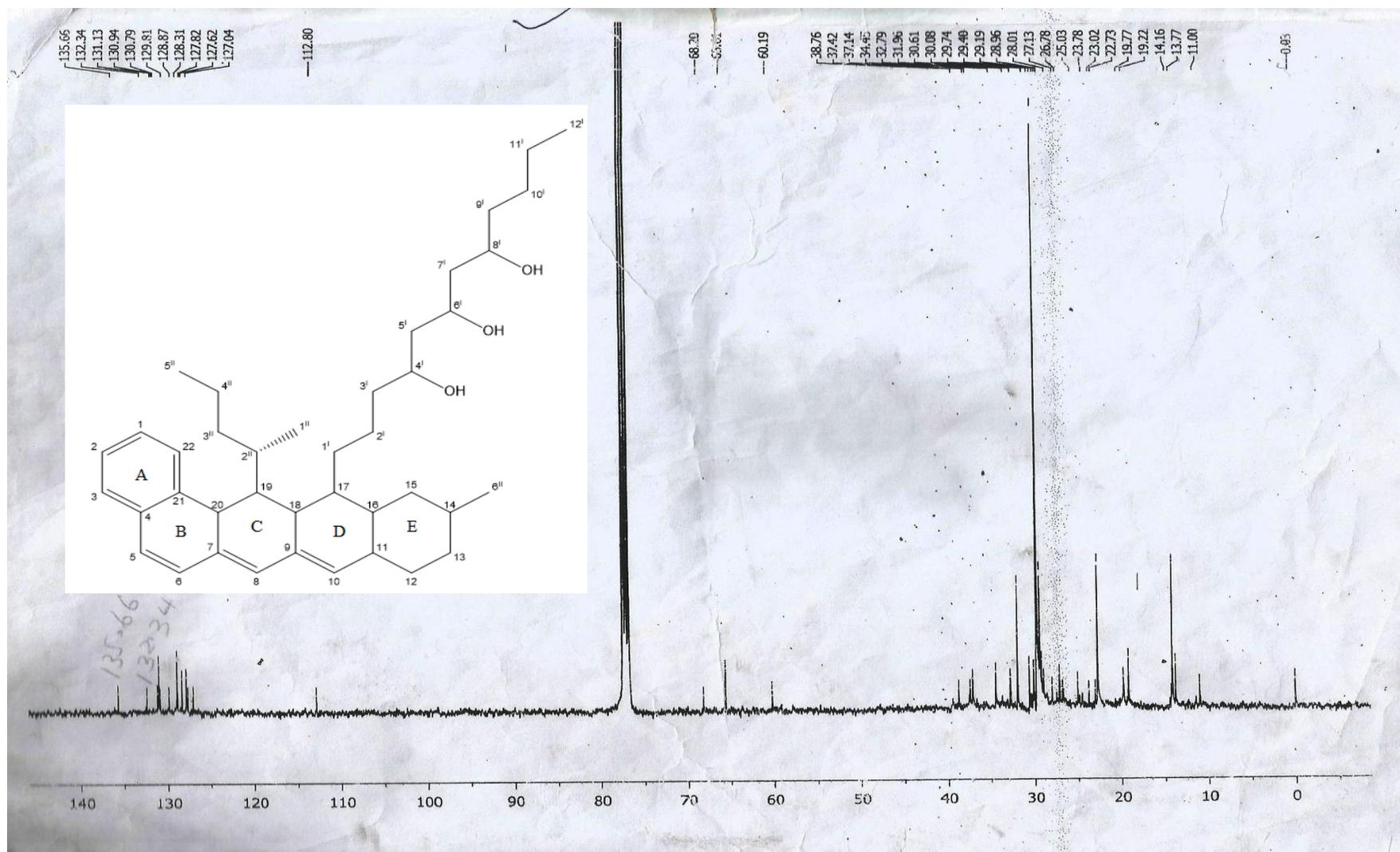
Figure 4.2: ^{13}C NMR Spectrum Analysis of Compound PEG3c

14.1.12.2 Proton NMR of compound PEG3c1

The ^{1}H NMR of compound PEG3c1 is summarized in Table 4.11.

Table 4.11: Summary of ^{1}H-NMR spectral data of compound PEG3c1

Position	^{1}H (ppm)	Assignment	Lit. values (ppm)
H-1^I	1.09	-CH$_2$ (multiplet)	1.25
H-2^I	1.09	-CH$_2$ (multiplet)	1.25
H-3^I	1.44	-CH$_2$ (multiplet)	1.45
H-4^I	4.10	-CH$_2$ (multiplet)	3.21
H-5^I	1.58	-CH$_2$	1.59
H-6^I	4.10	-CH$_2$ (multiplet)	3.21
H-7^I	1.58	-CH$_2$	1.59
H-8^I	4.10	-CH$_2$ (multiplet)	3.21
H-4^{1I}, H-6^{1I}, H-8^{1I}	4.15	CH$_2$-OH	3.58
H-9^I	1.45	-CH$_2$	1.44
H-10^I	1.09	-CH$_2$	1.25
H-11^I	1.43	-CH$_2$ (doublet)	1.33
H-12^I	0.90	H$_3$-C (multiplet)	0.90
H-1	7.27	-CH$_2$ in cyclohexadiene	7.28
H-2	7.26	-CH$_2$ in cyclohexadiene (doublet)	7.22
H-3	7.38	-CH$_2$ in a cyclohexadiene	7.33
H-5	7.73	-CH$_2$	7.91
H-6	7.14	-CH$_2$	6.58
H-8	7.04	-CH$_2$ in cyclohexadiene (multiplet)	6.19
H-10	4.32	-CH$_2$ in cyclohexadiene (quartet)	5.61
H-11	2.26	-C-C-	2.11
H-12	1.60	-CH$_2$ in cyclohexane	1.56

Table 4.11 Cont'd

Position	^{1}H (ppm)	Assignment	Lit. values (ppm)
H-13	0.98	-CH	1.27
H-14	1.61	-CH in cyclohexane	1.61
H-15	0.96	$-CH_2$	1.24
H-16	0.98	-C-C-	1.45
H-17	1.70	$-CH_2$	1.62
H-18	2.28	-C-C-	1.93
H-19	2.26	$-CH_2$	1.91
H-20	4.13	-C-C-	3.44
H-22	7.26	$-CH_2$ in a cyclohexadiene	7.24
H-1^{II}	0.96	$-CH_3$ (multiplet)	0.96
H-2^{II}	1.72	-CH	1.64
H-3^{II}	1.09	$-CH_2$ (multiplet)	1.25
H-4^{II}	1.43	$-CH_2$	1.33
H-5^{II}	0.92	$-CH_3$ (multiplet)	0.90
H-6^{III}	0.94	$-CH_3$ (multiplet)	0.96

Source: Laurie Starkey, Organic Chemistry Lab CHM 318L ^{1}H NMR Chemical Shifts
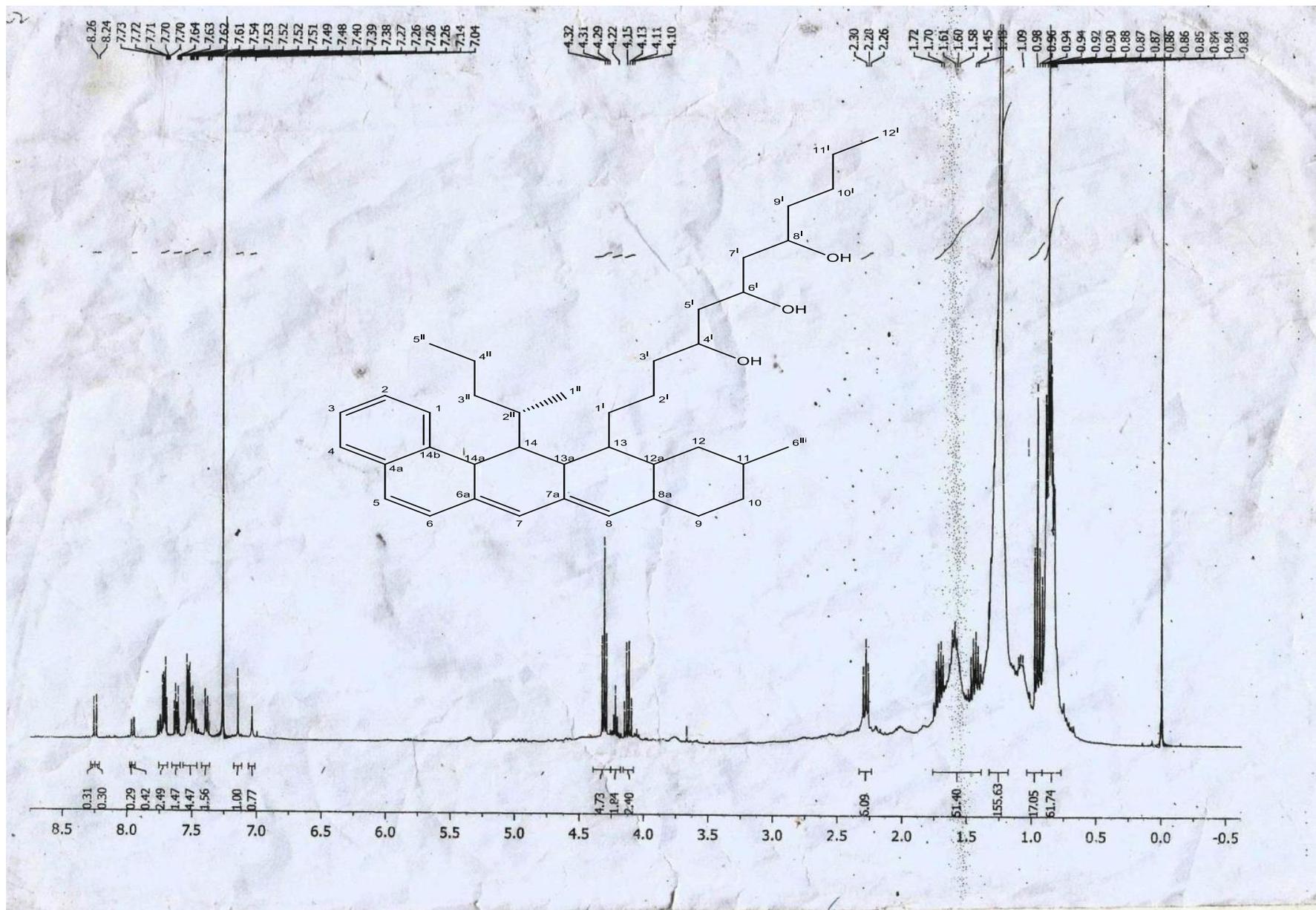
Figure 4.3: ^{1}H NMR Spectrum Analysis of Compound PEG3c1

4.1.12 TLC of fraction PEG7 filtrates

Washing of fraction PEG7 in petroleum ether severally, yield soluble and insoluble sub-fractions coded PEG7a to PEG7c. Their solvent system, spots on TLC and R_f values are shown in Table 4.12.

Table 4.12: TLC Profile of Sub-fractions PEG7 Petroleum Ether Filtrates

Pet. ether soluble	Solvent system	Number of spots on TLC	R_f value
PEG7a	$CHCl_3$ 100%	2	0.5
			0.54
PEG7b	$CHCl_3$ 100%	2	0.5
			0.58
PEG7c	$CHCl_3$ 100%	2	0.42
			0.48
	CH_2Cl_2 100%	3	0.32
			0.36
			0.45

4.1.13 GC-MS analysis of chloroform crude extract of *E. gilletii* roots

GC-MS analysis of the chloroform crude extract (CEG) revealed the presence of twenty one compounds which has been classified as shown in Table 4.13.

Table 4.13: GC-MS Analysis of Phytochemical Compounds Present in Chloroform Extract of *Ensete gilletii* Roots

Chemical Classes	Name of Compounds	Molecular Formula	Retention Time	Area %
Hydrocarbons	alpha-Dodecene	$C_{12}H_{24}$	8.126	2.31
	alpha-Tetradecene	$C_{14}H_{28}$	10.636	4.54
	2,8-Dimethylundecane	$C_{13}H_{28}$	12.927	1.17
	1-Nonadecene	$C_{19}H_{38}$	14.845	10.35
	3,7-Dimethyldecane	$C_{12}H_{26}$	14.902	2.11
	(3E)-3-Octadecene	$C_{18}H_{36}$	17.847	3.97
	9E)-9-Hexacosene	$C_{26}H_{52}$	19.791	10.31
	Tetrapentacontane	$C_{54}H_{110}$	19.550	4.73
	Nonane, 5-methyl-5-propyl-	$C_{13}H_{28}$	16.691	2.32
	Bicyclo[2.2.1]heptane,2-methyl	C_8H_{14}	17.842	2.99
	Decane, 2,3,8-trimethyl	$C_{13}H_{28}$	19.087	0.93
Fatty acids/alcohols	Pentadecyl alcohol	$C_{15}H_{32}O$	12.856	7.62
	3,5-Dimethyl-4-octanone	$C_{10}H_{20}O$	15.816	0.61
	1-(+)-Ascorbic acid 2,6-dihexadicane	$C_{38}H_{68}O_8$	16.38	08.77
	Phthalic acid, cyclobutyl tridecyl est	$C_{25}H_{38}O_4$	16.467	0.96
	1-Octadecanol, methyl	$C_{19}H_{40}O$	16.645	11.51
	Octadecanoic acid	$C_{18}H_{36}O_2$	18.041	2.99
	1-Heptacosanol	$C_{27}H_{56}O$	18.285	11.38
	Palmetic acid. beta-monoglyceride	$C_{19}H_{38}O_4$	20.677	1.37
	Methoxyacetic acid, 4-tridecyl ester	$C_{16}H_{32}O_3$	20.532	1.78
	Phenol, 2,4-di-tert-butyl-	$C_{14}H_{22}O$	12.055	7.28

4.1.14 Antibacterial Studies

Antibacterial efficacy of all three extracts (PEG, CEG and MEG) of *E.gilletii* was determined against selected organisms using disc diffusion method in comparison with reference standard (ampicillin). Their susceptibility test, MIC and MBC are presented in Figures 4.4 – 4.6.
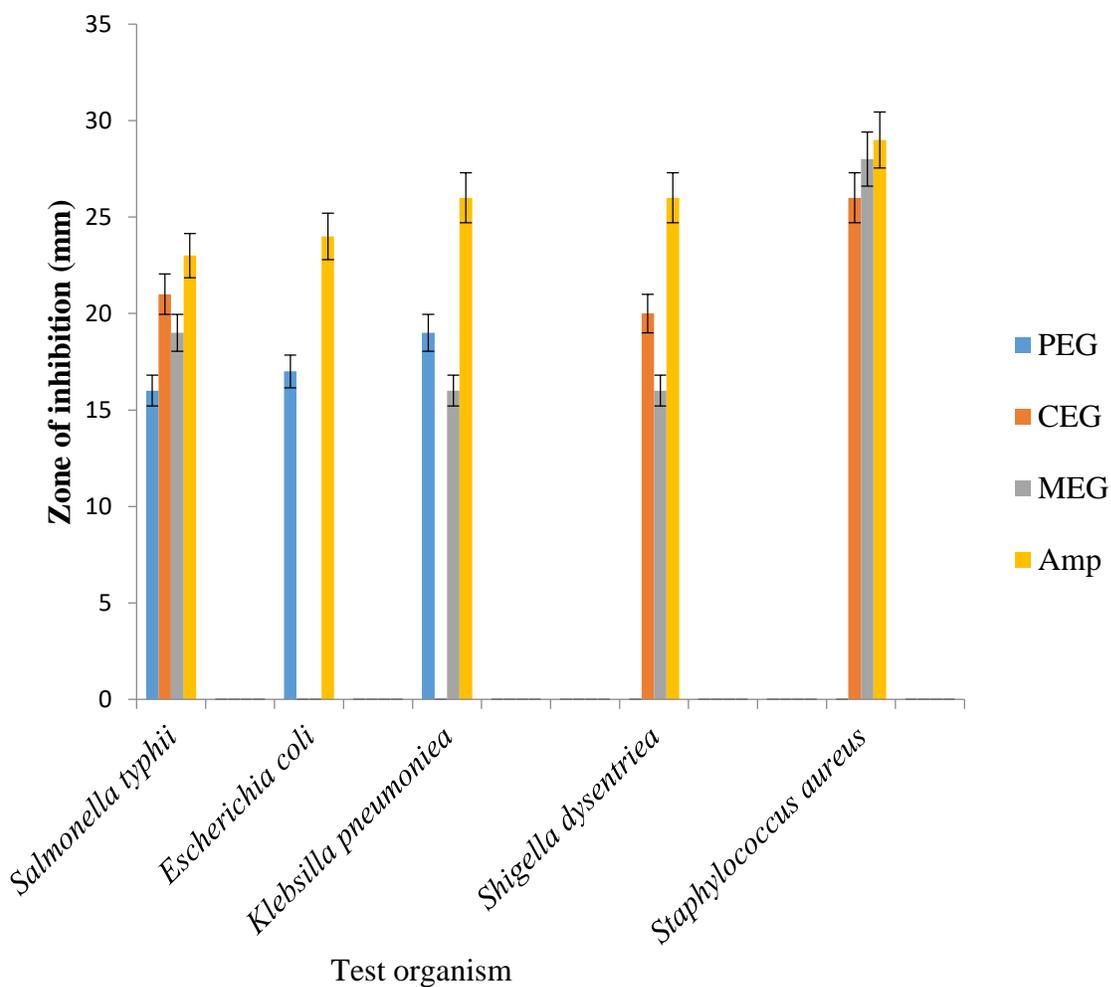

Figure 4.4: Susceptibility Test of Extracts in Comparison with Ampicillin against Selected Organisms

Keys: PEG= petroleum ether crude extract

CEG= chloroform crude extract

MEG= methanol crude extract
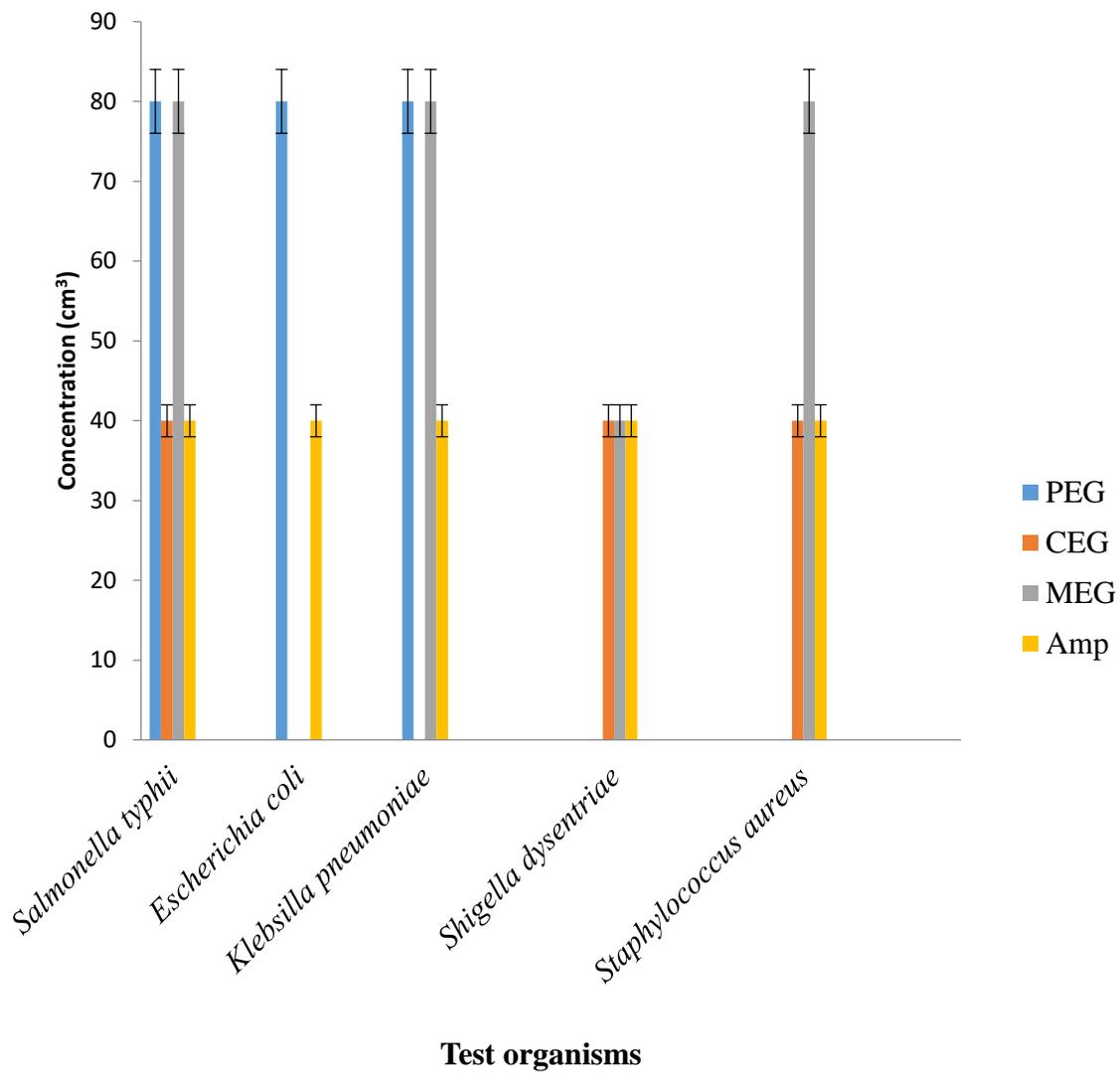
Amp= ampicillin

Figure 4.5: Minimum Inhibitory Concentration (MIC) of the three Extracts in Comparison with Ampicillin against Microbes

Keys: PEG= petroleum ether crude extract

CEG= chloroform crude extract

MEG= methanol crude extract
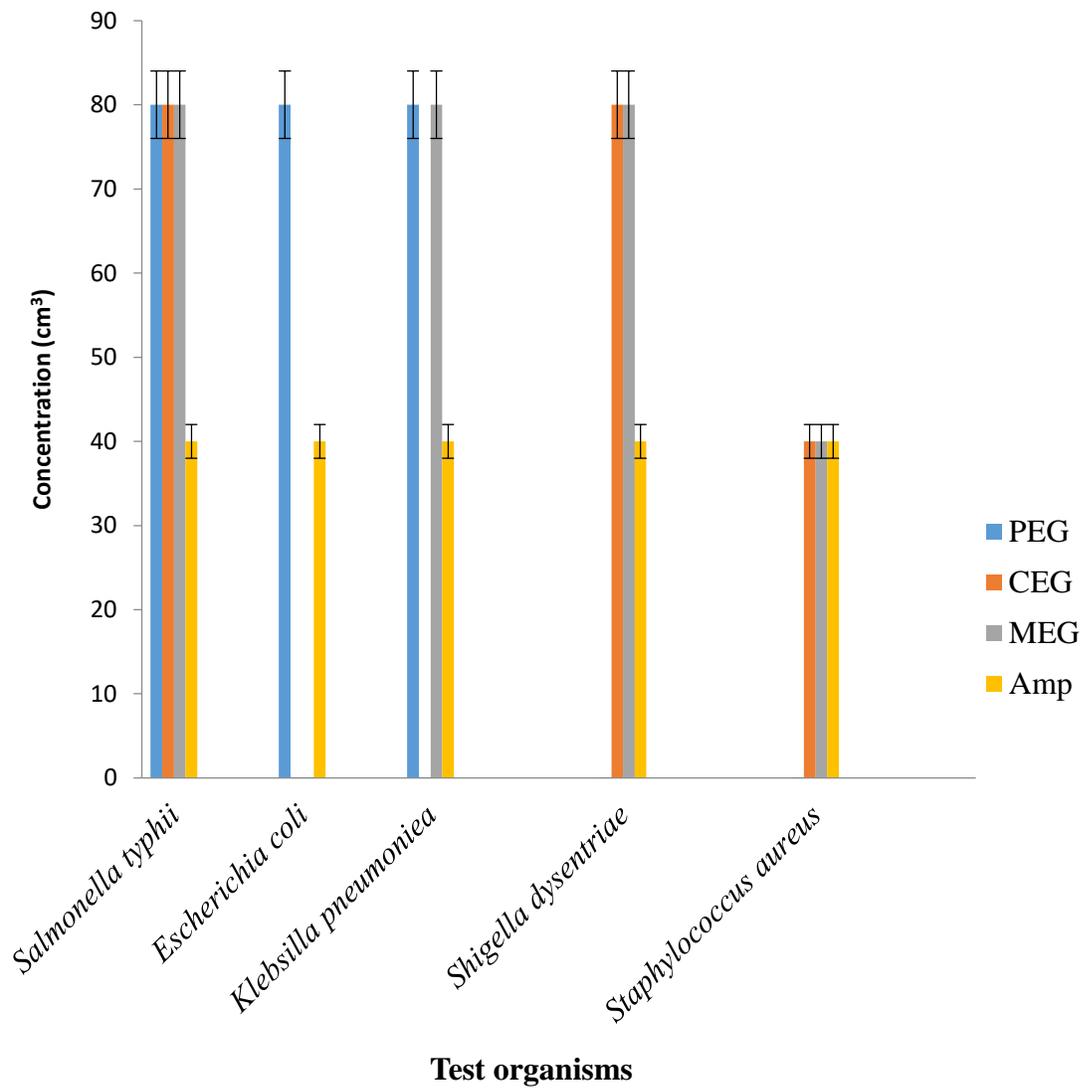
Amp= ampicillin

Figure 4.6: Minimum Bactericidal Concentration (MBC) of the three Extracts in Comparison with Ampicillin against Microbes

Keys: PEG= petroleum ether crude extract

　　　CEG= chloroform crude extract

　　　MEG= methanol crude extract

　　　Amp= ampicillin

4.2 Discussion of Results

4.2.1: % Extractable and physical appearance of crude extracts

The exhaustive and successive method employed for the extraction of phytoconstituents from the roots of *E.gilletii*, using petroleum ether, chloroform and methanol is a means of separating the non-polar constituents from the mid-polar, eventually the polar constituents. Several researchers have adopted this method of extracting phytoconstituents from plant samples using various organic solvents successively in either cold or hot percolation. The physical appearance of each extract is as a result of the solvent of extraction. Petroleum ether is a de-fattening solvent, which is commonly used for extracting lipids and oils (Handa *et al.,* 2008). The percentage extractable of the crude extracts, petroleum ether (PEG), chloroform (CEG) and methanol (MEG) extracts of *E. gilletii* revealed that methanol extract (MEG) had a higher percentage extractable of 8.5% as shown in Table 4.1. This indicates that the plant is rich in polar constituents. Methanol is reported as a general solvent that dissolves most secondary metabolites in plants and enhances the release of these phytoconstituents from plant tissue, it extracts polar and non-polar constituents. Several researchers have reported a maximum yield with methanol (Mythili and Ravindhran, 2012; Jamuna *et al.,* 2014). The petroleum ether (PEG) and chloroform (CEG) extracts had little difference in % yield, which could be due to their relatively close polarity. However, the petroleum ether extract (PEG) was 0.4% higher than the chloroform extract (CEG). The differences in their weight could be attributed to the physical nature of each extract.

4.2.2 Qualitative screening of *E.gilletii* roots extracts

The phytochemical screening of the petroleum ether (PEG), chloroform (CEG) and methanol (MEG) extracts revealed the presence of flavonoids, phenols, steroids, tannins, terpenoids,

saponins and glycosides as shown in Table 4.2. These phytochemical constituents are known for their bioactivities which includes; anti-microbial, anti-inflammatory, anti-pyretic, anti-diabetic, anti-oxidant, therapeutic and analgesic effects (Hodek *et al.,* 2002; Derek *et al.,* 2018). Phenols and terpenoids were abundant in all the extracts and are known to be good anti-microbial, anti-inflammatory, anti-pyretic, anti-septic agents. Petroleum ether and chloroform have been reported as good solvents for extraction of non-polar constituents such as terpenoids (Prashant *et al.,* 2011). Alkaloids were not confirmed in all three extracts. Flavonoids, glycosides, phenols, saponins, tannins, terpenoids and steroids were present in the methanol extract. Afolayan *et al.* (2014), also, reported the presence of same constituents in the plant seed. The tannins and flavonoids are known to have curative activity against several pathogens and therefore could be used for the treatment of various illnesses. Phenols are reported as anti-tumor agents and exhibit antioxidant properties (Usman and Osuji, 2007). The presents of similar constituents in plants belonging to same family (*Musacea*) have been reported.

4.2.3 Quantitative screening of *E.gilletii* root

The dried pulverised roots of *E.gilletii* yielded high saponins content (913.34 mg), compared to phenols (157.64 mg), flavonoids (33.16 mg), alkaloids (31.63 mg) and tannins (19.15 mg) as shown in Figure 4.1. This indicates that the plant is rich in saponins, which are either glycosylated (contains sugar molecule) or non-glycosylated/aglycone. Saponins are reported to be a class of compounds which are glycosylated steroids, triterpenoids and steroid alkaloids. Triterpenoid saponins are surface active glycosides of triterpenes that possess a wide, biologically active group of terpenoids. Literature shows that saponins exhibit various biological and medicinal properties,

such as; hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral and anticancer (Akinyeye *et al.,* 2014). However, Table 4.2 revealed the absence of saponin in the petroleum ether (PEG) and chloroform (CEG) extracts, an indication that saponin is a highly polar compound. Saponins are polar constituent, as such, better extracted in aqueous medium (Handa *et al.,* 2008), a mixture of water in a polar solvent (Bimakr, 2010).

4.2.4 GC-MS analysis of petroleum ether crude extract of *E.gilletii* roots

Gas chromatography-mass spectrometry (GC-MS) analysis carried out on the petroleum ether crude (PEG) extract revealed the presence of eighty compounds which was classified into terpenes/terpenoids, fatty acids and their esters, esters, carbonyl, steroids, phenolic/alcoholic compounds and hydrocarbons as shown in Table 4.3. The result shows that majority of the compounds present in the extract are hydrocarbons and terpenes. The GCMS result corresponds to the qualitative phytochemical screening which also reveals the abundance of terpenes present in the extracts. p-cymene which is classified as a terpene is also an anthracene derivative found in crude hydrocarbon and naturally occurring in plants, was revealed to have the highest area % (3.00) as shown in Table 4.3. The classes of compounds present in the root extract could be attributed to the solvent of extraction. Petroleum ether is known to extract mostly non-polar constituents.

4.2.5 Fractionation of crude petroleum ether extract of *E.gilletii roots*

The fractionation of the petroleum ether extract (PEG) was done using column chromatography. The column fraction PEG7 has the highest percentage extractable of 28% as shown in Table 4.4, this could be due to the mid polar solvent (EtOAc) present in the solvent system (CHCl$_3$:EtOAc; 9:1), which eluted more constituents compared to the solvent system (PE: CHCl$_3$) with less polar solvent. Several researchers have used ethyl acetate as a solvent for fractionation, with EtOAc fraction having a higher percentage yield (Akinyeye *et al.,* 2014). The difference in weights of the fractions could also be attributed to higher numbers of pooled fractions when compared to some fractions, which amounted to higher yield. From this result; it could be deduced that the higher the polarity increase, the more % yield.

4.2.6 TLC profile of pooled major fractions from column fractionation of petroleum ether extract (PEG)

Thin layer chromatography (TLC) profiles of seven major fractions presented in Table 4.5, shows that PEG3 and PEG7 have two better resolved spots with solvent system: CHCl$_3$ (100%), PE:EtOAc (3:1) as summarized in Table 4.6. Plates 4.1 and 4.2 showing TLC chromatograms of PEG3 and PEG7 respectively, confirms that the spots were better resolved with higher concentration of constituents present in PEG3. However, the spots were at a very close R$_f$ values (R$_f$ 0.56, 0.58) which required further fractionation for a better resolution of the two close spots. PEG3 also showed a positive colour to Liebermann Burchard's test, this confirms the presence of terpenes. The concentration of constituents present in fractions PEG3 and PEG7 was the basis for their selection and further purification.

4.2.7 Further fractionation of PEG3

Fraction PEG3 was further fractionated yielding three major sub-fractions coded (PEG3a-c). Their percentage yield revealed that the higher the polarity of the solvents of fractionation (PE:EtOAc) the higher the % yield as shown in Table 4.7. PEG3c have the highest yield of 44% with solvent system; PE : EtOAc (1 : 1).

4.2.8 TLC profile of 3 major sub-fractions of PEG3

The various TLC R_f values of re-fractionated sub-fraction of PEG3, reveals that PEG3c have better resolved spots with solvent system (CH_2Cl_2 100%; R_f 0.6, 0.8; PE: EtOAc 4:1; R_f 0.54, 0.6; hexane: EtOAc 5:1; R_f 0.45, 0.7) when compared to other sub-fractions, as shown in table 4.8 and Plate 4.4. PEG3c showed yellow colour on TLC which was observed with sunlight, iodine vapour and Chromogenic spray reagent (10% H_2SO_4). Based on the TLC profile and % yield, PEG3c was selected for further purification.

4.2.9 Preparative TLC of sub-fraction PEG3c

Chemical separations can be accomplished using high performance liquid chromatography (HPLC), by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Generally, the identification and separation of phytochemicals can be accomplished using isocratic system (using single unchanging mobile phase system). Gradient elution in which the proportion of organic solvent to water is altered with

time may be desirable if more than one sample component is being studied and differ from each other significantly in retention under the conditions employed.

Sub-fraction PEG3c was further purified using preparative thin layer chromatography (PTLC) in solvent system: CH_2Cl_2 (100%). The upper layer of the plate was scraped, washed in acetone. The resulting filtrate was dried in vacuo and coded PEG3c1 which was spotted on high performance thin layer chromatographic (HPTLC) plate, developed in solvent system: Hexane: EtOAC (5:1). Developed plate was sprayed with 10% H_2SO_4 and heat. Yellow coloured spot was observed with; (i) sunlight (ii) iodine vapour (iii) 10% H_2SO_4. Plate 4.6 shows developed chromatogram with R_f 0.52, 0.526. The yellow colour observed is reported as typical anthracene colour which is classified as terpene (**Furniss et al.,** 1989; Somashekar and Chetana, 2016).

4.2.10 Compound PEG3c1

The isolated compound (PEG3c1) was characterized using;

Physical: appearance of the isolate was observed to be yellow viscous liquid (10 mg) which is soluble in hexane and methanol with a weak aromatic odour. Anthracene oil was also reported to be a greenish-yellow to black oil (Collin *et al.,* 2006), soluble in water, hexane and methanol, with a weak aromatic odour (Haynes, 2011). TLC (R_f 0.52) of the isolate correspond with the reported R_f values of anthracene derivatives ranging from 0.4 -0.6. The isolated compound showed a positive colour to the anthracene test, with the appearance of yellow colour which indicates the presence of free anthracene.

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Spectral analysis: ^{13}CNMR spectrum of the isolated compound (PEG3c1) revealed 40 proton decoupled peaks. Several deshielded peaks were observed downfield (δ127.04 ppm- δ135.66 ppm) which are reported as carbons of aromatic compound, this indicates the presence of several phenylic (arylic) carbons as reported in literature. The peak at δ135.66 ppm is observed to be the least shielded peak. The peaks observed upfield (δ11.00 pp- δ38.76 ppm) are due to shielding effect from an electronegative molecule. The peaks observed upfield is showing quaternary aliphatic carbons, tertiary, secondary and a highly shielded peak at (δ0.03 ppm), which is the most shielded observed further upfield. Peaks within (δ6.0 ppm-6.9 ppm) in the ^{13}CNMR chemical shift data base are reported as carbons bearing OH group. This corresponds to the 3 peaks observed at (δ60.1 ppm, 65.0 ppm, 68.2 ppm) in the ^{13}CNMR spectrum; it indicates the presence of hydroxyl group. Table 4.10 shows the summary of the ^{13}C NMR spectrum.

The 40 conjugated carbon atoms observed, correspond with most anthracene derivates reported to have high carbon density (O'Neil, 2013), and tetraterpenes which are also reported to have 40 carbon units. A compound reported as anthracene derivative with chemical formula $C_{38}H_{38}$ was isolated and synthesized (Yicai *et al.,* 2019).

Several deshielded peaks observed at δ7.04 ppm- 8.26 ppm in the ^{1}H NMR spectrum, indicates the presence of several phenylic proton. The clustered peaks downfield show the compound is rich in phenyl protons; this corresponds to reported literature values. The peaks upfield (δ0.83 ppm- 1.72 ppm) are as a result of shielding effect from an electronegative molecule (oxygen). The multiplet peaks upfield in the ^{1}HNMR spectrum could be as a result of poor resolution. Table 4.11 shows the proton NMR data summary.

The GC-MS analysis of the crude PEG extract revealed the presence of these compounds; p-cymene, psi-cumene, cumene p-ethyl- which are reported as anthracene based compounds (Charleton *et al.,* 2016; Bartnik, 2017). Cumene and cymene are both yellow-liquid hydrocarbons which also occur as natural constituents belonging to terpene group of compounds. However, the GC-MS result did not reveal the isolated compound, which could be as a result of reactions the extract must have undergone during the process of purification, but the presence of compounds that are anthracene based confirms the possibility of the isolated compound to be a novel anthracene derivative.

4.2.10.1 Anthracene and its derivatives

Anthracene an ortho-fused arene and a member of acenes group, is a component of petroleum products consisting of fused benzene rings, reported as a colorless solid or green to yellow oil of polycyclic aromatic hydrocarbon with formula $C_{14}H_{10}$ (*Skalamera et al., 2017).* Most anthracene derivatives are reported to have high carbon atoms (O'Neil, 2013). Antracenes is used in the production of dyes, plastic scintillator, insecticides, light emission stabilizer and several other non-pharmaceutical purposes. However, varieties of anthracene derivatives having hydroxy group are reported to be pharmacologically active (Carl- Hermann, 2009; **Somashekar** and Chetana, 2016; Waller and Anthony, 2018). Dithranol is an anthracene derivative that impairs DNA replication and decreases cell division and is effective for healing psoriatic plaques (Waller and Anthony, 2018). Alizarin which is anthraquinone (derived from anthracene) derivative isolated from Madder root (*Rubia tinctorum, Rubiaceae*), was reported to be used as food coloring and also for chelating properties in the prevention of kidney stones (Bartnik, 2017); the anti-biotic properties was also

reported (Carl-Hermann, 2009). Anthracyclines (anthracycline antibiotics) are used in cancer chemotherapy. These anthracyclines inhibit DNA and RNA synthesis and prevents rapidly-growing cancer cells (Chiriboga, 2003). Several DNA binding and cleavage studies revealed that anthracene derivatives are good intercalator of DNA; it is adopted in certain anticancer drugs. Several amino methyl anthracene derivatives are reported as promising anticancer agents. Some anthracene derivatives are reported to be used as laxatives (Somashekar and Chetan, 2016).

4.2.10.2 Terpenes/Terpenoids

Terpenes belong to a class of natural hydrocarbon secondary metabolites and basically consist of five carbon isoprene units which are linked together in various ways, giving rise to a rich diversity of structural classes, with novel skeletons continuously discovered in various plants. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. They are classified into hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterpenes (C_{25}), triterpenes (C_{30}), tetraterpenes/polyterpenes (C_{40}), depending on its isoprene unit (C_5)n (isoprene rule). These classes of compounds are known to have biological, pharmacological and therapeutic effect. They are widespread in nature, mainly in plants as constituents of essential oils. Studies have revealed that a large number of compounds (more than 30,000) have been identified, screened and isolated from various plants (Rolf and Eckehard, 2016).
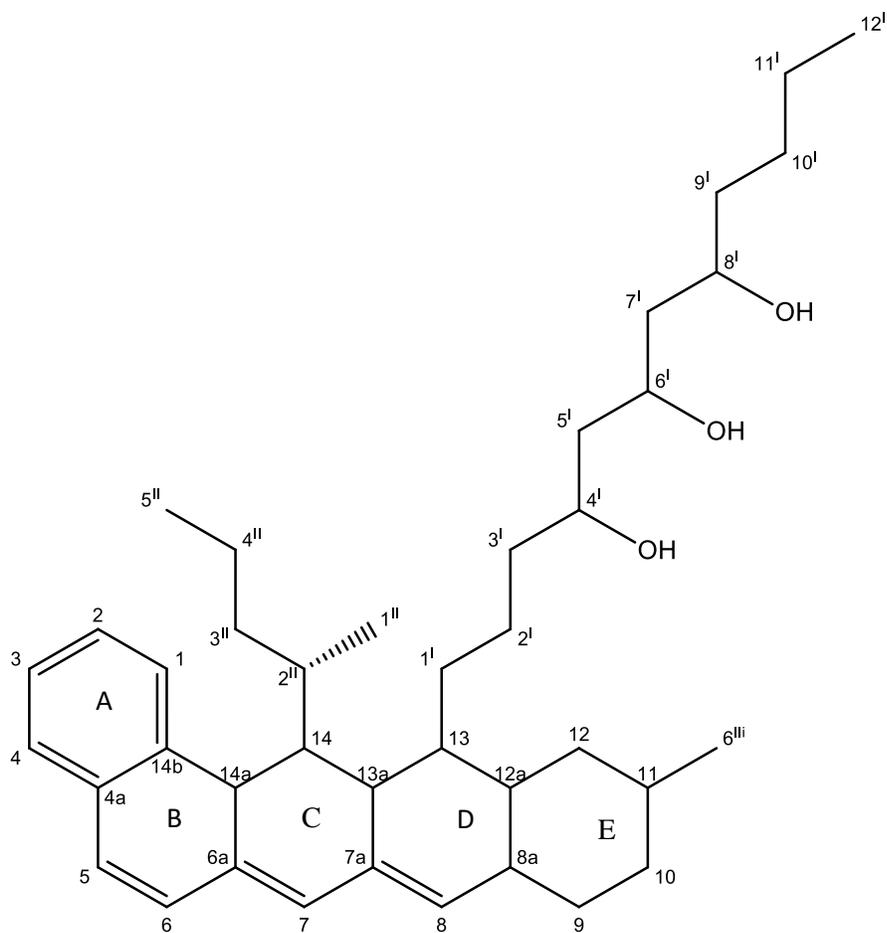
11-methyl-14-[(S)-pentan-2^{II}-yl)-13-[(8a,9,10,11,12,12a,13,13a,14,14a)-decahydrobenzo[a]

tetracen-1^{I}-yl)]dodecane-4^{I},6^{I},8^{I}-triol

Proposed Structure for the Isolated Compound (PEG3c1)

4.2.11 TLC of fraction PEG7 filtrates

Fraction PEG7 was washed in petroleum ether repeatedly. Each resulting filtrates coded (PEG7a-c) were analyzed with TLC and their profiles are shown in Table 4.12. From Table 4.12 which shows the TLC profiles of PEG7a-c petroleum ether filtrates, indicates that there was a separation and purification process as a result of the sub-fraction washed in solvent severally. Constituents that are not petroleum ether soluble were separated as residues, while the soluble was allowed to gel and re-washed in solvent. The process was monitored using TLC. PEG7a in solvent system ($CHCl_3$ 100%) showed 2 spots with close R_f (0.5, 0.54). As the procedure was repeated, the resulting filtrate in solvent system ($CHCl_3$ 100%) revealed 2 spots with similar Rf (0.5, 0.58). It was observed that there was a minor difference in PEG7a and PEG7b TLC profiles. The same procedure was repeated and PEG7c was obtained which further revealed a better resolution of the spots in solvent system ($CHCl_3$ 100%, Rf: 0.42, 0.48; CH_2Cl_2 100%, Rf: 0.32, 0.36, 0.45). It was observed that the solvent washing aided separation and purification. However, PEG7c revealed several resolved spots which could not further be purified as a result of minute yield.

4.2.12 GC-MS analysis of phytochemical compounds present in chloroform extract of *Ensete gilletii* roots

Gas chromatography-mass spectrometry (GCMS) result of the chloroform crude extract (CEG) revealed the present of 21 compounds. Table 4.13 shows classifications of the various compounds which are mainly hydrocarbons and fatty acids/alcohols. 1-Octadecanol, methyl ether was shown to have the highest area % (11.51) in close range with 1-Heptacosanol (11.38).

4.2.13 Antibacterial studies

The antibacterial susceptibility investigation reveals that the plant root extracts, petroleum ether (PEG), chloroform (CEG) and methanol (MEG) extracts were relatively potent against the selected test organisms, with the methanol (MEG) extract exhibiting a broad-spectrum activity when compared to a standard drug (Ampicillin) as shown in Figure 4.4. This could be attributed to the phyto-constituents present in the extracts which has antibacterial effect. However, the reference standard drug (Ampicillin) was more active compared to the extracts, this can be due to the refined nature of the drug with the active ingredients at a higher concentration compared to the 3 extracts. The petroleum ether (PEG), chloroform (CEG) and methanol (MEG) extracts were potent against *Salmonella typhii* with zones of inhibition at 16 mm, 21 mm and 19 mm respectively. Their MIC were 80 cm^3, 40 cm^3 and 80 cm^3 accordingly and their MBC at 80 cm^3 as shown in Figures 4.4 – 4.6. The chloroform (CEG) extract was found to have MIC at 40 cm^3 for all the test organisms which is the minimum as against 80 cm^3 for the petroleum ether and methanol extracts. Only the petroleum ether extract was active against *Escherichia coli* with zone of inhibition at 17 mm as against the 22 mm of the standard drug (ampicillin), with MIC and MBC at 80 cm^3. *E. gilletii* seeds extract was also reported to inhibit the growth of tested organisms (Afolayan *et al.,* 2014).

The proposed structure belongs to the class of benzo[a]tetracene derivative, particularly the bioactive nephthacene quinones which been known with antimicrobial (antibiotic) properties. For instance, many research works with related structure have been reported (Gomi *et al.,* 1988; Takeda *et al.,* 1988; Hofeditz *et al.,* 2018).

CHAPTER FIVE

5.0   CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the results, it revealed that the roots of *Ensete gilletii* have medicinal potentials which conform to the ethno-medicinal claims on the plant as reported by local practitioners. The phytochemical constituents such as; flavonoids, tannins, phenols, glycosides, terpenoids, saponins and phytosterols present in the plant extracts, accounts for its medicinal and antibacterial properties. The TLC profiles of the various sub-fractions show the presence of secondary metabolites that could be isolated and characterised from the plant roots.

Phytochemical studies of the PEG extract lead to the purification, characterisation of an anthracene derivative whose structure was elucidated using physical, chemical and spectral analysis. Using chromatographic techniques, the compound isolated (PEG3c1), was revealed to be an anthracene derivative.

All the extracts exhibited moderate activity against all tested organisms with MEG exhibiting a broad-spectrum activity. The plant can be a lead to the discovery of new and potent orthodox medicaments for the control and treatment of antibacterial infections.

5.2 Recommendations

Further work should be carried out on the roots of *Ensete gilletii* to isolate and characterise more of the active compounds.

The isolated compound (PEG3c1) should further be subjected to instrumental analysis such as; distortion enhancement polarisation transfer (DEPT), mass spectral analysis, to confirm the

structure of the compound. The isolated compound (PEG3c1) should further be tested for its antibacterial efficacy.

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