CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF FIVE FRUIT TREES AND NON-VOLATILE CONSTITUENTS OF *Theobroma cacao* L. POD-HUSK

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ABSTRACT

Essential Oils (EOs) are volatile secondary metabolites characterised by a strong odour and widely used for pharmacological and industrial applications. There is dearth of information on chemical compositions and bioactivities of EOs of some fruit trees in Nigeria. This study was therefore designed to extract and characterise the EOs from selected fruit trees, screen the EOs for bioactivity as well as to isolate and characterise non-volatile constituents from *Theobroma cacao* L. pod-husk due to its availability.

The plant samples (*Carica papaya* L., *Theobroma cacao* L., *Persea americana* M., *Ananas comosus* (L) Merr and *Chrysophyllum albidum* G. Don) were collected in Ibadan, identified and authenticated at the Herbarium of Forest Research Institute of Nigeria, Ibadan. Essential oils were extracted from the leaves, stem-barks, root-barks, fruits, peels, pod-husk and seeds of the plants using hydro-distillation method and analysed by Gas Chromatography (Flame Ionization Detector and Mass Spectrometry) techniques. The antibacterial activity of the EOs at 20 µg/mL was assayed on two Gram-positive and four Gram-negative bacteria using Microplate Alamar Blue Assay measured in UV/Visible spectrophotometer. The antioxidant activity of the EOs at 20 µg/mL was determined by radical scavenging procedure while insecticidal activity was evaluated by contact toxicity test using three grain pests. Pure compounds were isolated from methanol extract of *T. cacao* pod-husk by chromatographic techniques. The chemical structures of the compounds were elucidated using Infrared, Nuclear Magnetic Resonance and Mass Spectroscopic techniques. Data were analysed using descriptive statistics.

Twenty-seven EOs were obtained and their yield ranged from 0.1 to 1.2% (v/w). The major components in *P. americana* EOs were β-caryophyllene (12.7%; leaf), tetradecanal (31.8%; root-bark), globulol (25.4%; peel), (Z,Z)-4,15-octadecadien-1-ol acetate (21.8%; seed), tetradecanal (24.9%; stem-bark) and p-xylene (40.5%; fruit). *Carica papaya* EOs mainly comprised benzylisothiocyanate (89.1%; seed), octadecanol (62.5%; root), octadecanol (71.1%; stem), m-xylene (35.1%; stem-bark), heptadecanol (25.2%; fruit), phytol (21.8%; leaf), benzylisothiocyanate (71.5%; root-bark) and 9-hexadecen-1-ol (16.9%; peel). P-xylene (62.4%; fruit), p-xylene (29.9%; shoot) and tetradecanoic acid (8.6%; peel) dominated *A. comosus* EOs. The principal constituents in *T. cacao* EOs were hexadecanoic acid (78.7%; leaf), o-xylene (53.3%;

seed), ledol (33.6%; pod-husk) and β-bisabolol (17.3%; stem-bark). The dominant compounds in *C. albidum* EOs were m-xylene (66.7%; seed), p-xylene (21.4%; seed-bark), ethylhexadecanoate (19.9%; leaf), hexadecanoic acid (14.7%; stem-bark), m-xylene (53.1%; root-bark) and hexadecanoic acid (14.7%; fruit-bark). The chemical constituents for twenty-one of the EOs of the fruit plants were obtained for the first time ever. *Theobroma cacao* leaf EO exhibited the highest inhibition against Gram-negative bacteria at 78.6%, while *P. americana* fruit and peel EOs showed the highest inhibition against Gram-positive bacteria at 69.9%. *Persea americana* fruit and seed EOs displayed the highest and lowest radical scavenging activity at 42.1 and 1.2%, respectively. The EOs showed activity between 0 to 20% in insecticidal assay. Column chromatography of the methanol extract of *T. cacao* pod-husk yielded three known triterpenes: 24-hydroxy-9,19-cycloanost-25-en-3-one, stigmast-5-en-3β-ol and ergosta-5α,8α-epidioxy-6,22-dien-3β-ol.

The EOs have antibacterial and antioxidant properties which is indicative of their potential as sources of pharmaceuticals.

Keywords: Fruit tree, Essential oils, Antibacterial activity, Triterpenes, *Theobroma cacao* L.

Word count: 488

DEDICATION

This research work is dedicated to:

MY LOVING

DAD

&

MUM

A MESSAGE TO MY PARENTS

You are one in a billion couples;

For Parents like you are hard to come by

If you don't mind my saying it,

I owe everything in my life to you

And nothing to myself.

Your examples of hardwork, honesty,

Enthusiasm in others' progress and kindness

Encourage and spur me on

To hope for the really worthwhile things in life.

Your love inspires me.

Your guidance and support saw me through

The most trying periods of my life

To be factual, the greatest blessing of my life

Is that I have you as Parents.

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To others that I did not mention, and have contributed one way or the other to the success of this work, I want to say a big thank you.

Fatimah Temitayo ISHOLA

August, 2016

CERTIFICATION

We certify that this work was carried out under our supervision by **FATIMAH TEMITAYO ISHOLA** in the Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria.

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CHAPTER 1

INTRODUCTION

1.1 Fruits as Nutraceuticals

Nutraceuticals are substances that are regarded as food or part of food that provides medical or health benefits, for the prevention and treatment of diseases (De Felice, 1995). They include a broad range of categories such as dietary supplements, functional foods and herbal products (Radhika *et al*., 2011). The active compounds or phytochemicals in plants, especially fruits, have been associated with numerous health benefits (Lachance and Das, 2007) and are used as ingredients in many nutraceutical and pharmaceutical products today. Radhika *et al*., (2011) listed some sources of active ingredients from plants being used in manufacture of nutraceuticals.

Medicinal plants are of great importance to the health of individuals and communities with great potentials for pharmaceutical and nutraceutical applications. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non- nutritive chemicals that have protective or disease preventive properties. There are at least fourteen classes of secondary metabolites (phytochemicals) from fruits that exert biological activities and can potentially be used to promote human health. These include alkaloids, amines, cyanogenic glycosides, diterpenes, flavonoids, glucosinolates, monoterpenes, non-protein amino acids, phenylpropanes, polyacetylenes, polyketides, sesquiterpenes, tetraterpenes, triterpenes, saponins and steroids (Thompson and Thompson, 2010). Research by Mukherjee *et al.* (2011) highlighted some chemical compounds from various parts of plants that exhibit potential antioxidant activities, including madecossoside, asiaticoside, catechin, epicatechin, 4-hydroxycinnamic acid, esculetin, curcumin, xanthorrhizol, anthocyanins, diosgenin, gallic acid, ginsenoside, β-carotene and cyanidin-3-glucoside. However, plant extracts can be toxic with

excessive lethal constituents such as aristolochic acids, pyrrolizidone alkaloids, benzophenanthrine alkaloids, viscotoxins, saponins, diterpenes, cyanogenetic glycosides and furanocoumarins (Bahorun *et al*., 2008). These compounds can affect human health since nutraceutical products, unlike pharmaceutical products, are not as well regulated and are commonly consumed without supervision or medical guidance. On the other hand, phenolic compounds from a variety of fruits such as catechin, anthocyanins, quercetin, kaempherol, resvasterol, curcuminoids, genistein, apigenin, carotenoids, carnosic acid, caffeic acid and ferulic acid are known to possess antioxidant activities and a sun-protective effect against UV light-induced damage (Zaid *et al*., 2009).

Fruits are vital to humans. In fact, humans and many animals have become dependent on fruits as a source of food. They account for a substantial fraction of the world's agricultural output, and some (such as the apple and the pomegranate) have acquired extensive cultural and symbolic meanings (Lewis, 2002). Generally, fruits are high in fiber, water, vitamin C and sugars, although this latter varies widely from traces as in lime, to 61% of the fresh weight of the date (Braide *et al.*, 2012). Regular consumption of fruit is associated with reduced risks of cancer, cardiovascular disease (especially coronary heart disease), stroke, Alzheimer disease, cataracts and some of the functional declines associated with aging (Lewis, 2002). All these potentials give fruits the nutraceutical property.

1.2 Justification for the Research

Essential oils (EOs) or volatile oils are concentrated extracts characterized by a strong odor obtained from plants by distillation or cold pressing used as medicines by traditional healers. They represent a small fraction of a plant’s composition but confer the characteristic for which aromatic plants are used in the pharmaceutical, nutraceutical, food and fragrance industries (Anitescu *et al.*, 1997).

Essential oils are very interesting natural plant products and among other qualities they possess various biological properties. The term “biological” comprises all activities that these mixtures of volatile compounds (mainly monoterpenoids and sesquiterpenoids, benzenoids, phenylpropanoids) exert on humans, animals, and other plants. On account of the complexity of these natural products, the toxicological or

biochemical testing of an EO will always be the sum of its constituents which either act in a synergistic or in an antagonistic way with one another. Therefore, the chemical composition of the EO is very important for the understanding of its biological properties (Baser and Buchbauer, 2010).

The essential oils (volatile oils) from plants are known for their antisepticis, bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, which find uses in embalmment, preservation of foods and as antimicrobial, insecticidal, analgesic, sedative, anti-inflammatory, spasmolytic and locally anaesthetic remedies (Piccaglia *et al*., 1993; Shapiro *et al*., 1994; Xu *et al*., 2011). They have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons and oxygenated compounds which are responsible for the characteristic odors and flavors (Pourmortazavi and Hajimirsadeghi, 2007). The proportion of individual compounds in the oil composition is different from trace levels to over 90% (*δ*-limonene in orange oil). The aroma of oils is the result of the combination of the aromas of all components (Anitescu *et al.*, 1997).

Fruits present an increasing economic importance in tropical regions, especially in the field of eccentric juices' market. The flavor compositions of different medicinal plants especially fruits have already been described for guava, banana, mango, melon, papaya, passion fruit, pineapple, cupuaçu and bacuri (Maróstica and Pastore, 2007) and also for other Brazilian fruits (Augusto *et al.*, 2000; Franco and Shibamoto, 2000). Numerous scientific investigations also point at consecutive rich eco-friendly sources of immunostimulant, anticancer and antimicrobial properties, especially among fruits, but only few of them involve waste parts of the fruits, i.e. seeds and peels. Many of the fruits seeds and peels are thrown in the garbage or fed to livestock (Chanda *et al*., 2010; El-Hawari and Rabeh, 2014). This present study aims to compile the information available about the volatile compounds present in some Nigerian fruits in order to diffuse the importance of such fruits and to promote researches in this field.

Pods are the outer layer of some fruits which is hard in texture and sometimes too bitter or astringent to be eaten raw, as in the case of cocoa. They are usually discarded when consuming fruits. They are also called pericarps or rinds that surround the seeds (Azila and Azrina, 2012). The pericarp consists of three main parts, namely the epicarp

or exocarp, mesocarp and endocarp. The outermost part, the epicarp, is usually called the skin or peel of the fruits. The middle layer, mesocarp, can be edible in some fruits such as mango, or fibrous like in palm oil fruit. Finally, the endocarp encloses the seeds. It occurs in various forms, such as the hard shell of coconuts or the soft shell of cocoa (Bewley *et al*., 2006). In between the mesocarp and endocarp, there is also a part called the aril or placenta of the seed that can be consumed. This part is usually white in color and juicy as an attractant to animals in order for the plant to grow diversely (Hion *et al*., 1985). Representing the outer part of the fruits, the pericarp comes in various colors and changes during ripening depending on the types of fruits. For example, cocoa pods when ripened turn yellow from either red maroon or green.

Although pods or pericarps are usually discarded when consuming the edible parts of fruits, they contain some compounds that exhibit biological activities after extraction and make them a source of pharmaceutical and nutraceutical products (Azila and Azrina, 2012). Most fruit pods contain polyphenolic components that can promote antioxidant effects on human health. Additionally, anti-inflammatory, antibacterial, antifungal and chemopreventive effects are associated with these fruit pod extracts. Besides polyphenolics, other compounds such as xanthones, carotenoids and saponins also exhibit health effects and can be potential sources of nutraceutical and pharmaceutical components (Azila and Azrina, 2012). Information on fruit pods or pericarp of *Garcinia mangostana*, *Ceratonia siliqua*, *Moringa oleifera*, *Acacia nilotica*, *Sapindus rarak* and *Prosopis cineraria* has been presented and discussed with regard to their biological activity of the major compounds existing in them. The fruit pods of other ethno-botanical plants have also been reviewed (Azila and Azrina, 2012).

Cocoa and cocoa products have received much attention due to their significant polyphenol contents (Sabongi *et al*., 1998). Cocoa beans processing produce cocoa pod husks and pulps. Cocoa pod husk's wastes have not been optimized by the majority of cocoa farmers in Nigeria. They are sometimes used as animal feed, fertilizer, or discarded. According to literature, cocoa pod husks contain pectin component which are potential source of production of marketable natural pectin-derived emulsifier (Yapo and Koffi, 2013). However, there is dearth of information on the secondary metabolites present in the pod-husk waste.

Infectious diseases are leading cause of death worldwide. Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Because of increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action.

Extraction, identification and separation of the essential oil components of Nigerian fruit trees is another way of enhancing their economic value and industrial application, providing a cheaper and safer alternative source of raw material for industrial and other useful purposes, as well as providing a safer and cheaper means of waste management through transformation of fruit wastes to a source of industrial wealth.

1.3 Research Objectives

- To extract and characterise the essential oils from five fruit trees using GC and GC-MS for characterization.
- To determine the antibacterial, insecticidal and antioxidant activity of the extracted essential oils.
- To extract and screen the fruit trees for phytochemicals
- To isolate and characterise secondary metabolites from *Theobroma cacao* L. pod

CHAPTER 2

LITERATURE REVIEW

2.1 Chemical Constituents of Plants

The chemical constituents of plants can basically be categorized into two viz; the volatile and the non volatile. Volatile components in plants are typically classified into four major categories: terpenoids, fatty acid derivatives, amino acid derivatives and phenylpropanoid/benzenoid compounds. They are mostly found in the essential oils of the plants. Non volatile components are flavonoids, sugars, alkaloids, tannins, saponins, glycosides, steroids.

2.2 Plant Secondary Metabolites

Secondary metabolites are organic molecules that are not involved in the normal growth and development of an organism. Primary metabolites on the other hand, such as nucleic acids, amino acids, carbohydrate and fat have a key role in the survival of the species (Harborne, 2001; Wink, 2004), playing an active function in the photosynthesis and respiration, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability (Roze *et al.*, 2011), often playing an important role in plant defense (Anurag *et al.*, 2015). These roles include: protection against environmental stresses such as drought and excessive light radiation; inhibiting herbivores and other pathogen attacks; influence allellopathy and act as an attractant to pollinators (Hernandez *et al.*, 2004; Zobel *et al.*, 1999; Schreiner, 2005). Several of these metabolites have therapeutic properties and their concentration in the plant tissues is considered as the main factor to evaluate the therapeutic value and quality of a given herb (Wills *et al.*, 2000). They contain numerous natural products with interesting pharmacology activities (Verpoorte, 1998; Savithramma *et al.*, 2011).

A simple classification of secondary metabolites based on their biosynthetic origin includes three main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) and nitrogen or sulphur containing compounds (such as alkaloids and glucosinolates) (Croteau *et al.*, 2000). A number of traditional separation techniques with various solvent systems and spray reagents, have been described as having the ability to separate and identify secondary metabolites. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants (Thrane, 2001).

2.2.1 Alkaloids

Alkaloids are basic compounds synthesized by living organisms containing one or more heterocyclic nitrogen atoms, derived from amino acids (Kabera *et al.*, 2014), pharmacologically active (Aniszewski, 2007) and found in approximately 20% of the species of vascular plants (Hegnauer *et al.*, 1988). A huge variety of structural formulas, coming from different biosynthetic pathways and presenting very diverse pharmacological activities are characteristic of the group (Brielmann *et al.*, 2006). Most alkaloids are believed to function as defensive elements against predators, especially mammals because of their general toxicity and deterrence capability (Harborne, 1998; Hartmann *et al.*, 1991). Many are toxic and can cause death, even in small quantities but have a long history in medication, such as codeine **(2.1)** as an antidepressants (Simera *et al.*, 2010; Smith *et al.*, 2006; Vree *et al.*, 2000). Some interfere with components of the nervous system, especially the chemical transmitters; others affect membrane transport, protein synthesis and miscellaneous enzyme activities (Creelman and Mullet, 1997).

Alkaloids are usually colourless, crystalline, non- volatile solids which are insoluble in water, but are soluble in organic solvents. Some alkaloids are liquids which are soluble in water e.g. connine **(2.2)** and nicotine **(2.3)** and a few are coloured e.g. berberine **(2.4)** is yellow (Finar, 1997). Besides carbon, hydrogen and nitrogen molecules of alkaloids may contain sulphur and rarely chlorine or phosphorus (Lewis, 1998).

2.2.2 Phenolic Compounds

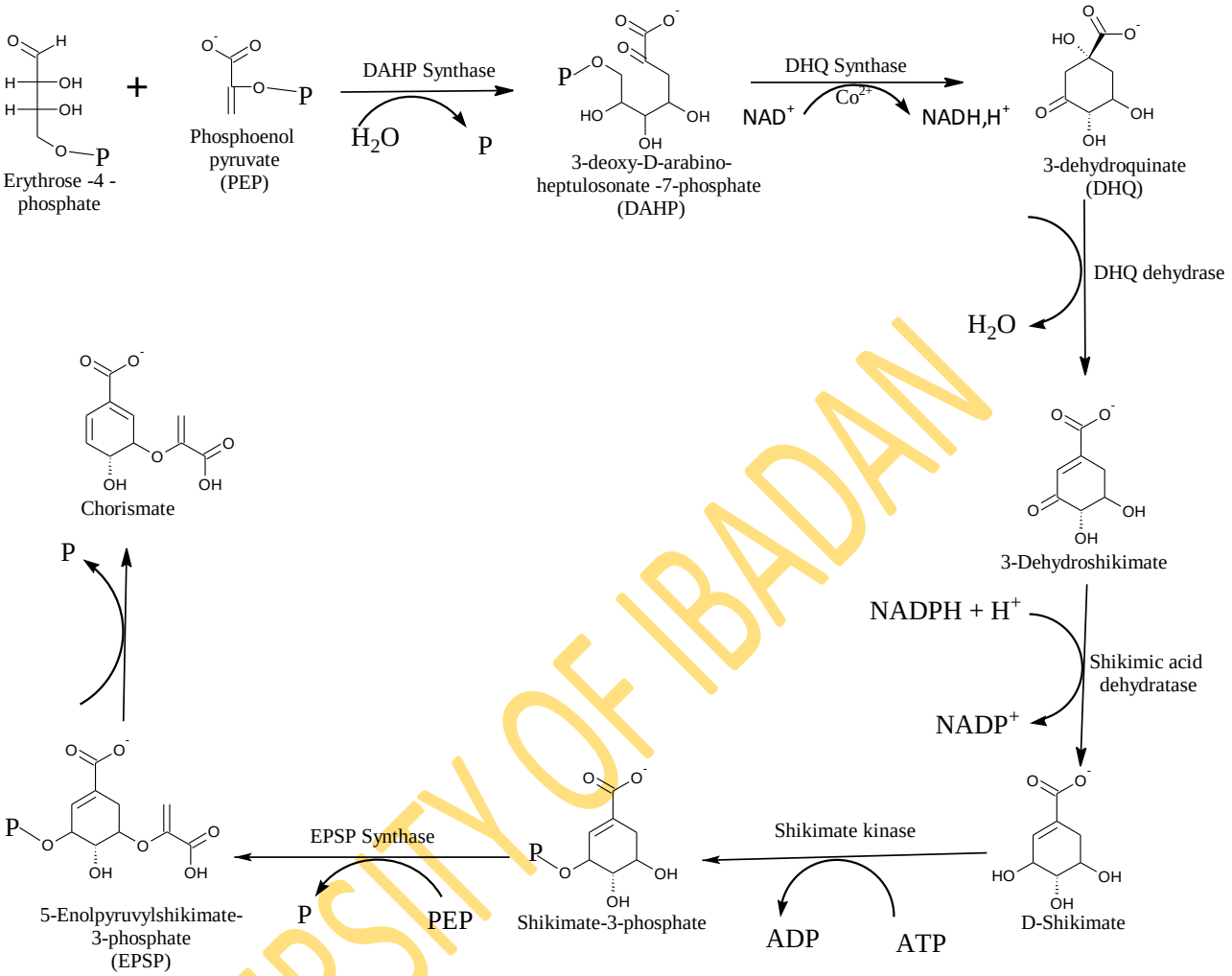
Phenolic compounds are one of the largest groups of secondary plants constituents synthesized by fruits, vegetables, teas, cocoa and other plants that possess certain health benefits. They usually possess an aromatic ring bearing one or more hydroxyl groups and range from simple phenolic molecules to highly polymerised compounds (Anurag *et al.*, 2015). Their biosynthetic origin is through the shikimate pathway (Scheme **2.1)** (Finar, 2000; Petrussa *et al.*, 2013). They are categorised into several classes based on the number of carbon atoms in the basic carbon skeleton. Table **2.1** shows some examples of the different classes of phenols with their basic carbon skeleton and the number of carbon atoms in the skeleton.

Phenolic compounds have antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties and may prevent oxidative stress (Park *et al.*, 2001).

2.2.3 Terpenoids

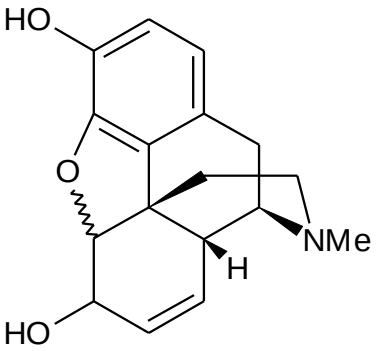
Terpenoids are the largest and most diverse family of secondary metabolites, ranging in structure from linear to polycyclic molecules and in size from the five-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units. A simple unifying feature of all terpenoids is that they are derived from a simple process of assembly of a C-5 unit, the isoprene unit C_5H_8 (Gershenzon and Dudareva, 2007). During their formation, the isoprene units are linked in head and tail fashion. The number of units incorporated into a particular terpene serves as a basis for their classification as shown in Table **2.2**. The classification of terpenoids ranges from essential oil components, the volatile, mono and sesquiterpenes (C_{10} and C_{15}), through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoids pigments (C_{40}) (Wang *et al.*, 2005). Each of these various classes of terpenoids is significant in either plant growth metabolism or ecology (Harbone, 1984).

Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, and the colour of yellow flowers.

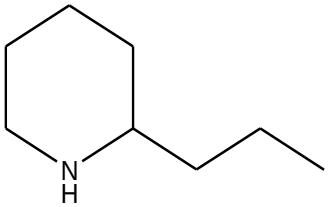


Scheme 2.1: The Shikimate pathway

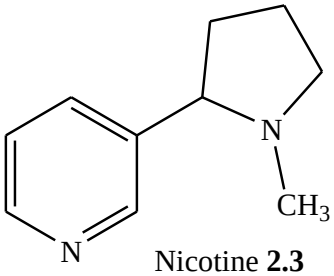
Source: Petrussa *et al.*, 2013



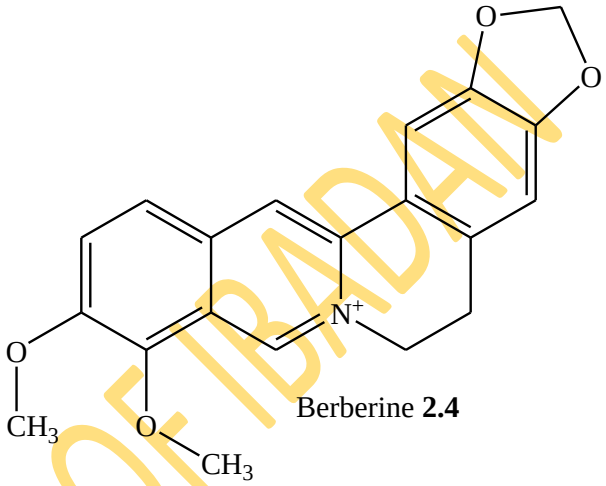
Codeine **2.1**



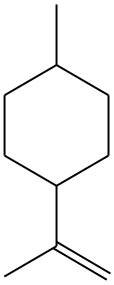
Coniine **2.2**



Nicotine **2.3**

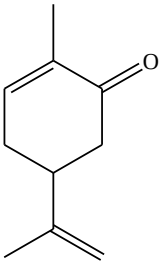


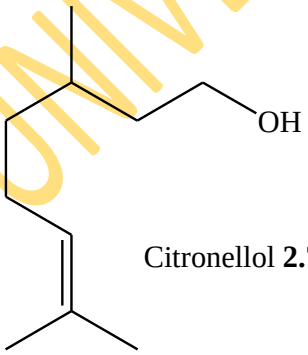
Berberine **2.4**



Limonene **2.5**

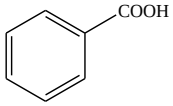
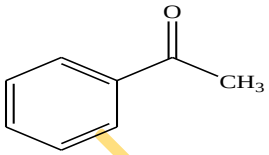
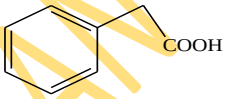
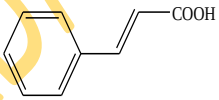
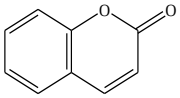
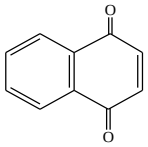
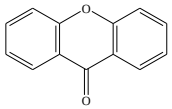
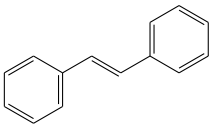
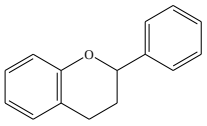
Carvone **2.6**





Citronellol **2.7**

Table 2.1: Examples of different classes of phenolic compounds

No. of C-atoms	Skeleton	Classification	Basic Structure
7	C_6-C_1	Phenolic acids	
8	C_6-C_2	Acetophenones	
8	C_6-C_2	Phenylacetic acids	
9	C_6-C_3	Hydroxycinnamic acids	
9	C_6-C_3	Coumarins	
10	C_6-C_4	Napthoquinones	
13	C_6-C_1-C_6	Xanthones	
14	C_6-C_2-C_6	Stilbenes	
15	C_6-C_3-C_6	Flavonoids	

Source: Harbone, 1999; Crozier *et al.*, 2006

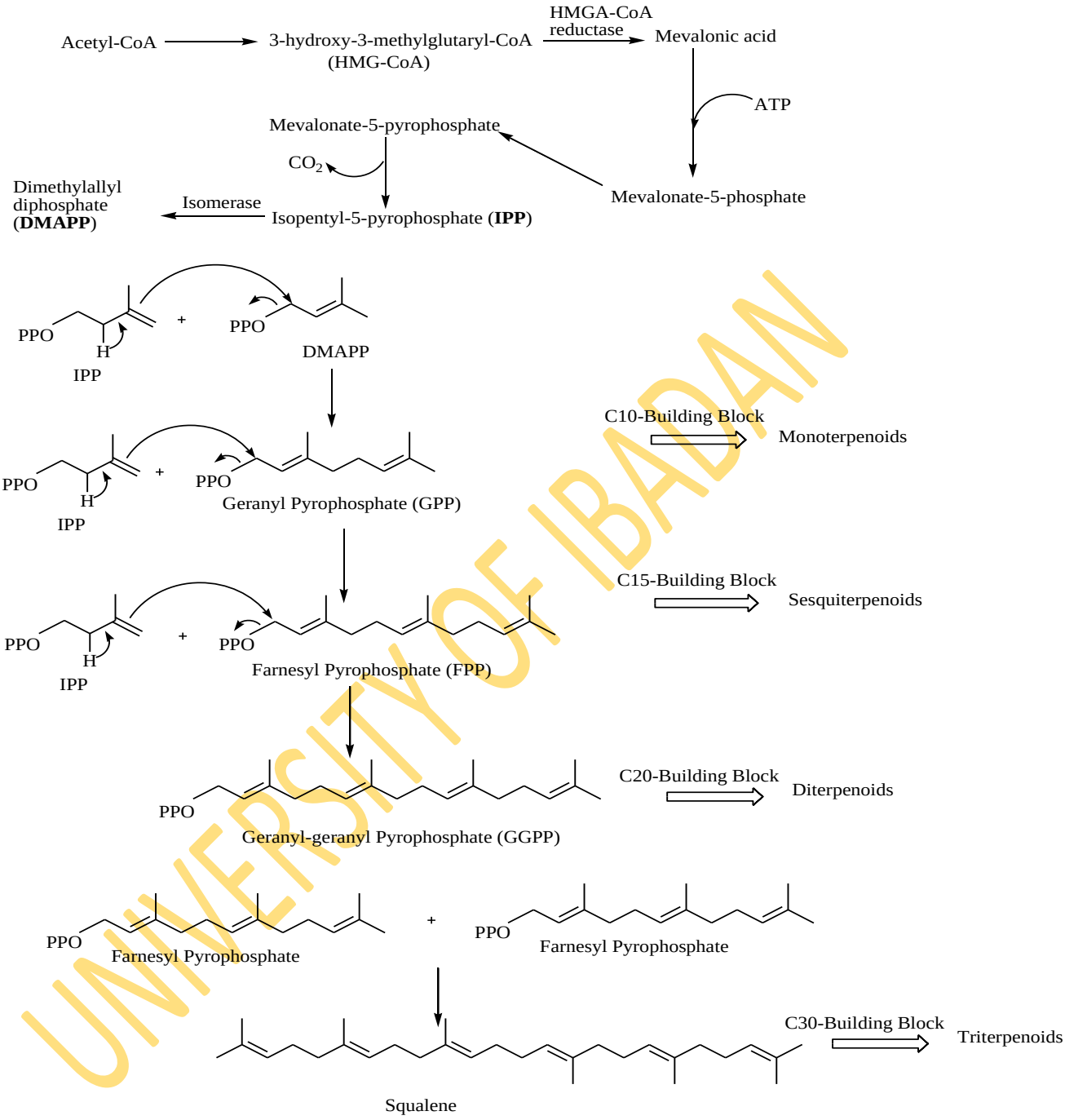
Table 2.2: Examples of different classes of terpenoids

No. of Isoprene units	No. of C-atoms	Terpenes	Example
1	5	Hemiterpenes	Prenol
2	10	Monoterpenes	Eucalyptol, Limonene
3	15	Sesquiterpenes	Farnesol, Farnesene
4	20	Diterpenes	Phytol, Gibberellin
5	25	Sesterterpenes	Geranylfarnesol
6	30	Triterpenes	Squalene
8	40	Sesquarterpenes	Ferrugicadiol, Carotenoids
>100	>500	Tetraterpenes	Rubber, Cytokonines

Source: Kogan *et al.*, 2006

2.2.3.1 Biosynthesis of terpenoids

Terpenoids biogenetically originated through the condensation of the universal phosphorylated derivative of hemiterpene, isopentenylpyrophosphate (IPP) $CH_2C(CH_3)CH_2CH_2OPP$ and dimethylallylpyrophosphate (DMAPP) $(CH_3)_2C\ CH\ CH_2OPP$. In biosynthesis, a molecule of isopentenylpyrophosphate (IPP) and dimethylallylpyrophosphate, which are biosynthesized from three acetylcoenzyme A moieties through mevalonic acid (MVA) via the mevalonate pathway (Kuzuama and Seto, 2003), are linked together to give geranylpyrophosphate (GPP), which on addition of another IPP unit forms farnesylpyrophospahte (FPP). GPP and FPP are precursors of monoterpenes and sesquiterpenes respectively (Finar, 2000; Rohdich *et al.*, 2001; Paul, 2002). Thus, terpenoids biosynthesis is based on the isoprene molecules $CH_2C(CH_3)CHCH_2$, their carbon skeletons are built up from the union of two or more of these C-5 units. The classification of terpenoids ranges from essential oil components, the volatile, mono and sesquiterpenes (C_{10} and C_{15}), through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoids pigments (C_{40}). The biosynthesis of terpenoids is shown in Scheme **2.2**.



Scheme 2.2: The mevalonic acid pathway

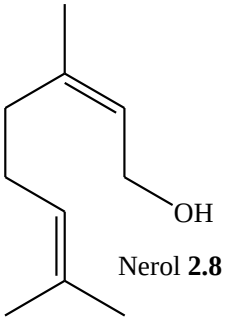
Source: Rohdich *et al.*, 2001

2.2.3.2 Pharmacological relevance of terpenoids

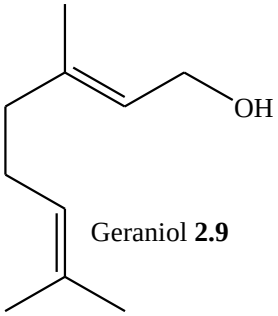
Terpenoids are used extensively for their aromatic qualities. Extensive biological investigations have been carried out within the group and these studies have revealed a broad spectrum of pharmacological and physiological properties (Maffei, 2010). They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Recent findings demonstrate that certain nitrogenous terpene derivatives possess the potent anti-hypertensive activity and may indicate a new era in medicine through the synthetic terpenoids path (Kabera *et al.*, 2014). The antimicrobial and insecticidal properties of other terpenoids have led to their utilization as pesticides and fungicides in agriculture and horticulture (Kataev *et al.*, 2011; Böhme *et al.*, 2014).

Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities in mammary tumor models and thus may represent a new class of therapeutic agents. Limonene **(2.5)** for example has been an interesting target molecule for chemists and biologists (Duetz *et al.*, 2001). Limonene is a well-established chemopreventive and therapeutic agent against tumor cells (Kris-Etherton *et al.*, 2002; Crowell, 1999; Fabian, 2001). Carvone **(2.6)** has also been shown to prevent chemically induced lung and forestomach carcinoma development (Wattenberg *et al.*, 1989). The mechanism of action of monoterpenes against tumor cells is the induction of apoptosis and interference of the protein prenylation of key regulatory proteins (Crowell, 1999; Fabian, 2001; Ariazi *et al.*, 1999; Crowell *et al.*, 1991). Acyclic monoterpenes, citronellol **(2.7)**, nerol **(2.8)** and geraniol **(2.9)**, have been reported to exhibit activity against *Mycobacterium tuberculosis* (Rajab *et al.*, 1998). Eucalyptol **(2.10)** is an ingredient in many brands of mouthwash and cough suppressant. It controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition. Although eucalyptol is used as an insecticide and insect repellent, it is one of many compounds that are attractive to males of various species of orchid bees, who apparently gather the chemical to synthesize pheromones. The anti-inflammatory activities of some medicinal plants result from the presence of one or more sesquiterpene lactones. Artemisinin **(2.11)** is an important sesquiterpene lactone with

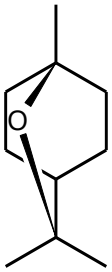
highly potent antimalarial activity (Abdin *et al.*, 2003; Bez *et al.*, 2003; Haynes, 2001; Brossi *et al.*, 1988; Robert *et al.*, 2002; Delabays *et al.*, 2001). Parthenolide **(2.12)**, another sesquiterpene lactone found in *Tanacetum parthenium, Leucanthemum parthenium* and *Pyrethrum parthenium*, is responsible for the majority of the medicinal properties of traditional herbal remedy. Chamazulene **(2.13)**, α- Bisabolol **(2.14)** are terpenoids isolated from *Matricaria chamolilla* and are commonly used for the treatment of skin inflammation and as antibacterial and antifungal agents (Dewick, 2002). Phytol **(2.15)** is one of the simplest and most important acyclic diterpenes. Trans-phytol, isolated from *Lucas volkensii* exhibits significant antituberculosis activity (Rajab *et al.*, 1998). Taxines **(2.16)**, a cyclic diterpene, isolated from *Taxus baccata* have been well studied because of their anticancer activity (Gogas and Fountzilas, 2003). Pleuremutillin **(2.17)** a diterpenoid from some species of mushroom (Novak and Shlaes 2010, Sreedhar *et al.*, 2009) and 12- demethylmulticauline **(2.18)** also a diterpenoid from *Salvia multicaulis* (Ulubelen *et al.*, 1997) have been reported to have antimycobacterial activity. Several triterpenoids have been found to be of pharmacological relevance. Friedelin **(2.19)** is reported to exhibit antifeedant, anti-cancer, antiinflammatory, anticonvulsant, antidysentery and antiulcer activities (Sharma *et al.*, 2009). Ursolic **(2.20)** and oleanolic **(2.21)** acids and their derivatives have been reported to exhibit antitumour (Tokuda *et al.*, 1986), gastroprotective (Astudillo *et al.*, 2001), and antimicrobial activities (Woldemichael *et al.*, 2003) with the 28-ester derivatives exhibiting weak antimicrobial effect (Weimann *et al.*, 2002). The antimycobacterial activity of oleanolic acid and its naturally occurring derivatives have been reported (Jim´enez-Arellanes *et al.*, 2003). Betulinic **(2.22)**, ursolic and oleanolic acids and derivatives have been found to inhibit viral replication (Ma *et al.*, 2002; Kashiwada *et al.*, 1998). Lanostane–type **(2.23)** triterpenes have been isolated from several species of mushrooms such as *Astraeus pteridis*. The compound has good inhibitory activity against *M. tuberculosis* (Stanikunaite *et al.*, 2008).



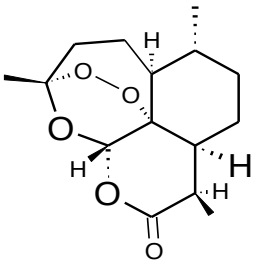
Nerol **2.8**



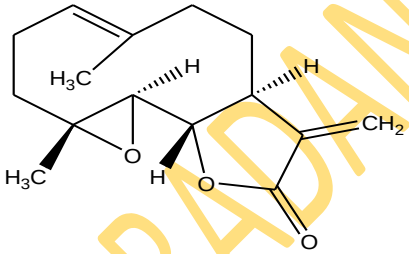
Geraniol **2.9**



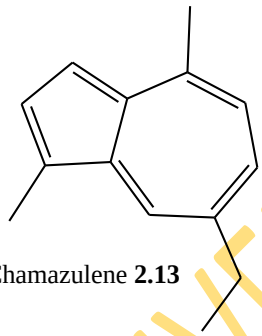
Eucalyptol **2.10**



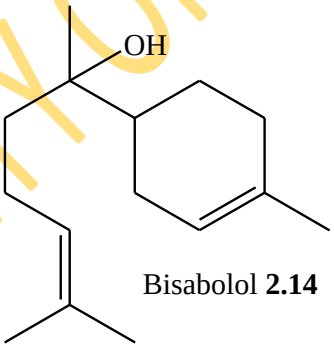
Artemisinin **2.11**



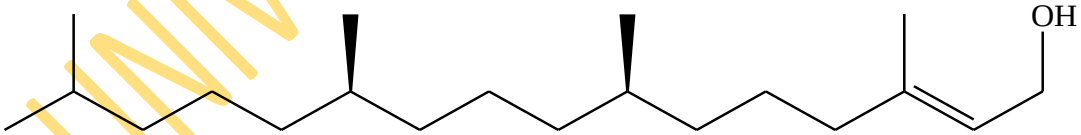
Parthenolide **2.12**



Chamazulene **2.13**



Bisabolol **2.14**



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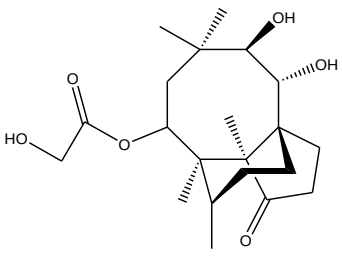
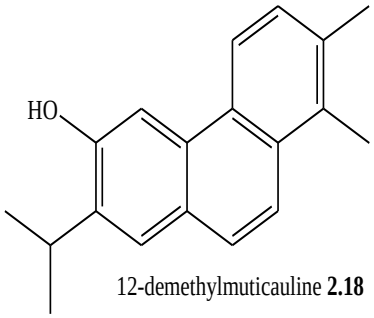
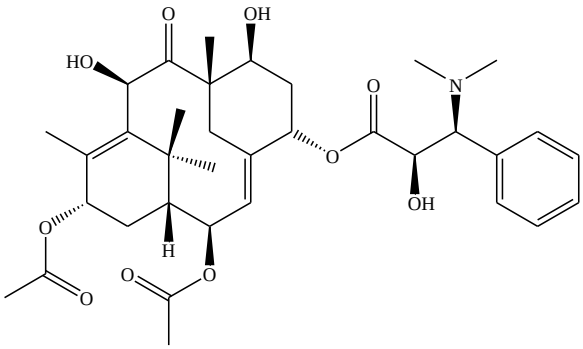
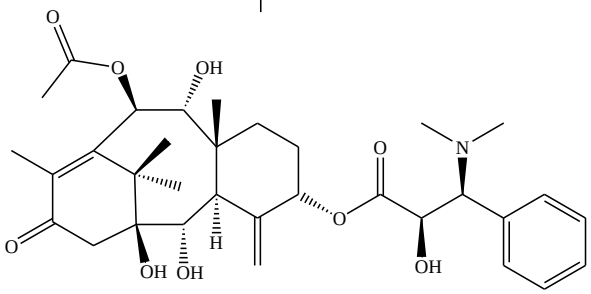


Pleuremutillin **2.17**

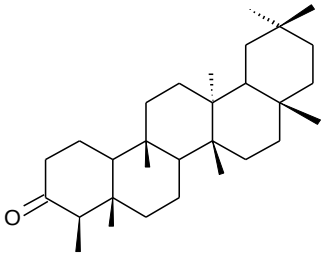


12-demethylmuticauline **2.18**

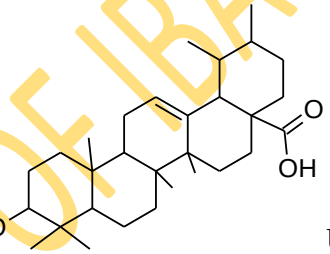




Taxine A and B **2.16**

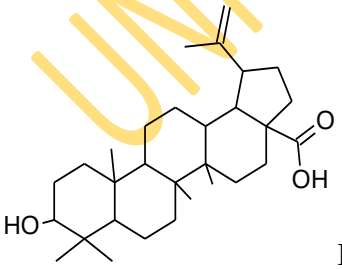


Friedeline **2.19**

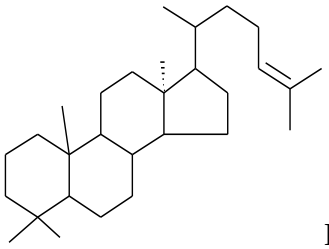


Ursolic acid **2.20**

Oleanolic Acid **2.21**



Betulinic Acid **2.22**



Lanostane **2.23**

2.2.3 Steroids

Sterols are triterpenes which are based on the cyclopentane perhydrophenanthrene ring system. They share the same biosynthetic origin with the terpenoids by acetate pathway through the cyclization of squalene (Harborne, 1998; Finar, 2000). At one time, sterols were mainly considered to be animal substances, but in recent years an increasing number of such compounds have been detected in plant tissues. Plant steroids are called 'phytosterols' which include; β-sitosterols **(2.24)**, stigmasterol **(2.25)** and campesterol **(2.26)**. Phytosterols occurred in higher plants as free and simple glycosides.

The occurrence of ergosterol **(2.27)** is confined to lower plants like yeast and many fungi. Fucosterol **(2.28)** which is the main steroid of many brown algae occurs mainly in lower plants but also appears occasionally in higher plants. It has also been detected in coconut (Harborne, 1998).

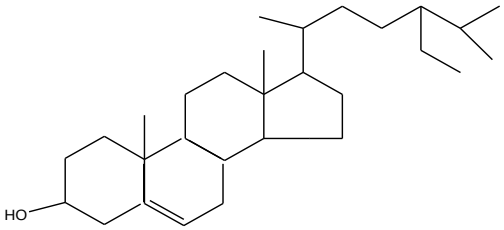
Pharmacologically, steroids have been shown to exhibit hormonal and anti-inflammatory activities. They are used as contraceptives, androgenic and anabolic agents. They also exhibit antifungal, antibacterial, antiviral and hypolipidemic activities (Saeidnia *et al.*, 2014).

2.2.4 Saponins

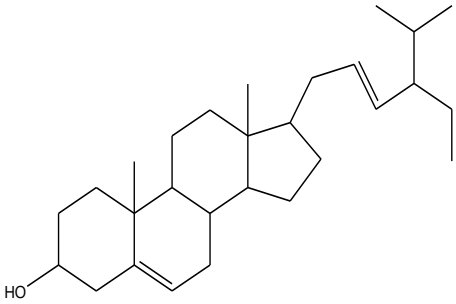
Saponins are plant glycosides of both triterpenes and sterols. They are surface-active agents with soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells. Sapogenins are the aglycone of saponins and are characterized by the presence of a spiroketal side chain. A characteristic property of saponin is the haemolysis caused by an intravenous injection of their aqueous solutions into animals; these solutions are comparatively harmless when taken orally (Finar, 1997).

The search in plants for saponins has been stimulated by the need for readily accessible sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance, like cortisone and contraceptive estrogen. Saponins are also of economic interest because of their occasional toxicity to cattle, as found in saponins of alfalfa or their sweet taste as found in glycyrrhizin of liquorice root (Ogundajo, 2014). Several reports have shown that saponin exhibit a wide range of pharmacological properties. Simoes-Pires *et al.* (2005) reported the use of saponins as vaccine adjuvants

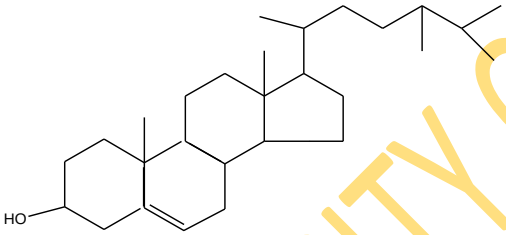
in the treatment of the Herpes Simplex Virus (HSV), Human Immuno-deficiency Virus (HIV), and influenza.



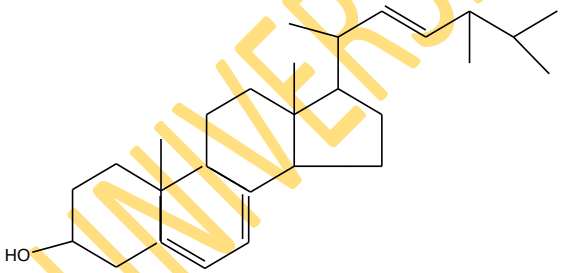
β-sitosterols **2.24**



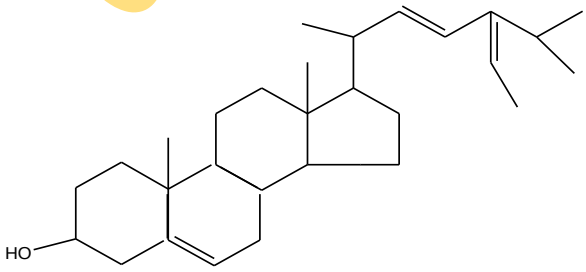
Stigmasterol **2.25**



Campesterol **2.26**



Ergosterol **2.27**



Fucosterol **2.28**

2.3 Extraction of Secondary Metabolites

Many approaches can often be employed to extract secondary metabolites, although water is used as an extractant in many traditional medicines. Organic solvents of varying polarity are generally selected through modern methods of extraction to exploit the solubility of various plant constituents. Obviously, wrong choice of solvent and method will cause the entire processes to fail or the desired compounds from the material may not be released from the matrix completely. Some extraction procedures usually applied for the extraction of natural products from plants are:

2.3.1 Cold Method

2.3.1.1 Percolation

In percolation, the powdered plant materials are soaked initially in a solvent in a percolator (a cylindrical or conical container with tap at the bottom). Additional solvent is then added on top of the plant material and allowed to percolate slowly drop-wise out of the bottom of the percolator. In this method, filtration of the extract is not required because there is a filter at the outlet of the percolator. Percolation is adequate for both initial and large scale extraction. The extent to which the material is ground can influence the extract yield. Hence, fine powder, resins and plant materials that swell excessively (e.g. those containing mucilage) can clog the percolator. Furthermore, if the material is not homogeneously distributed in the container, the solvent may not reach all the areas and the extraction will be incomplete. A disadvantage of the technique is that large volumes of solvents are used and this can be time consuming (Cannel, 1998).

2.3.1.2 Maceration

This method is simple and still widely used. The procedure involves soaking the pulverized plant materials in a suitable solvent in a closed container at room temperature. The technique is suitable for both initial and bulk extraction. Occasional or constant stirring of the preparation (using mechanical shaker or mixers to guarantee homogenous mixing) can increase the extraction yield. The extraction ultimately stops when equilibrium is attained between the concentration of metabolites in the extract and that of the plant material. After extraction, the residual plants material (marc) has to be separated from the solvents. This involves a rough clarification by decanting, which is usually followed by a filtration. To ensure exhaustive extraction, it is common to carry out an initial maceration, followed by clarification and an addition of fresh

solvent to the residue. This can be performed periodically with all the filtrates pooled together. It is a batch method of extraction (Harbone, 1998).

The major drawback of this technique is the fact that the process can be quite time consuming, taking from a few hours up to weeks (Takahashi *et al.*, 2001). Exhaustive extraction can also consume large volumes of solvent and can lead to potential loss of metabolite and, or plant materials. Furthermore some compounds may not be extracted efficiently if the compounds of choice are poorly soluble at room temperature in the solvent used. The major advantage of the method is those compounds that are thermo labile are not affected.

2.3.2 Hot Method

Soxhlet extraction is the widely used hot method in the extraction of plant metabolites because of its convenience. This method is adequate for both initial and bulk extraction. The plant powder is packed in a thimble in an extraction chamber, which is placed on top of a collecting flask. A suitable solvent is added to the flask, and the flask is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath. As the solvent saturates the plant material in the flask, it will solubilize the metabolite which is emptied into the flask. Fresh solvent is re-condensed and the material extracted in the thimble continuously. It is usually a continuous method of extraction (Harbone, 1984). This makes Soxhlet extraction less time and solvent-consuming than maceration or percolation. However, the main disadvantage of soxhlet extraction is that the extract is constantly heated at the boiling point of the solvent used, and this can damage thermo labile compounds and, or initiate the formation of artifacts (Zygmunt and Namiesnik, 2003).

In recent years, several faster and more automatic extraction techniques for solid samples have been replacing conventional techniques. Among the modern techniques are extraction by supercritical fluids extraction (SFE), pressurized liquids extraction (PLE), Ultrasound–Assisted solvent extraction and microwave assisted extraction MAE). These alternative techniques considerably reduce the consumption of solvents, increase the speed of the extraction process, and simplify it (Ali *et al.*, 2007). The major drawback on these techniques in this part of the world is the lack of steady power supply; hence the conventional techniques are still the methods of choice for this project.

2.4 Essential Oil

Essential oils, volatile oils or simply the "oil" of the plant from which they were extracted, such as “oil of lemongrass” are hydrophobic liquids containing volatile aroma compounds extracted from vegetal materials using various extraction techniques (Yazdani *et al.*, 2011). From the view point of practical applications, these materials may be defined as odiferous bodies of an oily nature, obtained almost exclusively from vegetable organs: flowers, leaves, barks, woods, roots, rhizomes, fruits, and seeds (Burt, 2004; Celiktas *et al.*, 2006; Skocibusic *et al.*, 2006; Chalchat and Ozcan, 2008; Hussain *et al.*, 2008; Anwar *et al.*, 2009a). These oils have strong aromatic components that give a plant its distinctive odor, flavor, or scent (Koul *et al.*, 2008).

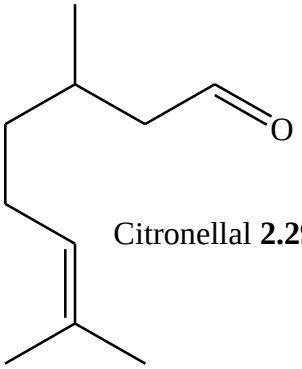
Essential oils (EOs) are very interesting natural plant products and among other qualities they possess various biological properties viz antibacterial, antifungal, antioxidant and anti-carcinogenic properties (Tzortzakis, 2007) that these complex mixtures of a large number of constituents (mainly terpenoids, benzenoids, phenylpropanoids, etc.) in variable ratios (Van Zyl *et al.*, 2006) exert on humans, animals, and other plants.

Essential oils are secondary metabolism products in plants. Plants have essential oil components and quality varying with geographical distribution, harvesting time, growing conditions, and extraction method (Yang *et al.*, 2005). These oils are typically liquid at room temperature and are easily transform from a liquid to a gaseous state at room temperature or a slightly higher temperature without decomposing (Koul *et al.*, 2008). Presently, essential oils are most often used in the food industry for flavoring, the cosmetic industry for fragrances, and the pharmaceutical industry for their functional properties. However, dozens of plant essential oils have been screened for fumigant toxicity against a variety of insect pests primarily for agricultural and food storage (Wang *et al.*, 2006; Ayvaz *et al.*, 2008; Benzi *et al.*, 2009; Ebadollahi *et al.*, 2010).

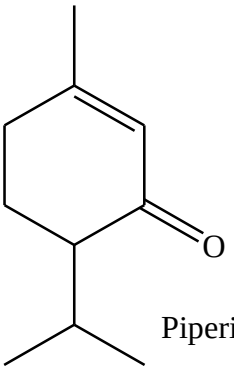
Essential oils are very complex natural mixtures. The components include two groups of distinct biosynthetical origin; the terpenoid group which is the main group and the non-terpenoid group which may contain short-chain aliphatic substances, aromatic substances, nitrogenated substances, and substances with sulphur (Croteau *et al.*, 2000; Bowels, 2003). In essential oils, the two terpenoid groups, the monoterpenes and sesquiterpenes allow for a great variety of structures. Depending on the functional

group attached they can be: aldehydes like citronellal **(2.29)**, ketones such as piperitone **(2.30)**, esters for example linalyl acetate **(2.31)**, oxides like 1,8-cineole **(2.32)**, alcohols such as menthol **(2.33)**, phenols for example thymol **(2.34)** and hydrocarbons like limonene **(2.5)** (Koul *et al.*, 2008).

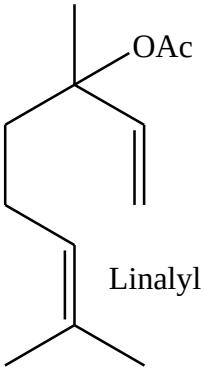
Essential oils exhibit a wide spectrum of pharmacological activities such as infection control, wound healing, pain relief, anti-nausea, anti-inflammation and anti-anxiety (Halcon, 2002; Kalemba and Kunicka, 2003). Traditional medicines containing essential oils have been scientifically proven to be effective in treating various ailments like malaria and others of microbial origin (Lopes *et al.*, 1999; Nakatsu *et al.*, 2000; Goulart *et al.*, 2004). The oils or some of their constituents are indeed effective against a large variety of organisms including bacteria (Holley and Dhaval, 2005; Basile *et al.*, 2006; Schelz *et al.*, 2006; Hu'snu'Can Baser *et al.*, 2006), virus (Duschatzky *et al.*, 2005), fungi (Hammer *et al.*, 2002; Velluti *et al.*, 2003, 2004; Serrano *et al.*, 2005; Cavaleiro *et al.*, 2006; Pawar and Thaker, 2006; Soylu *et al.*, 2006), protozoa (Monzote *et al.*, 2006), parasites (Moon *et al.*, 2006; Priestley *et al.*, 2006), larvae (Hierro *et al.*, 2004; Pavela, 2005; Morais *et al.*, 2006; Amer and Mehlhorn, 2006a,b; Ravi Kiran *et al.*, 2006), worms, insects (Bhatnagar *et al.*, 1993; Lamiri *et al.*, 2001; Liu *et al.*, 2006; Burfield and Reekie, 2005; Yang and Ma, 2005; Sim *et al.*, 2006; Kouninki *et al.*, 2005; Park *et al.*, 2006a,b; Chaiyasit *et al.*, 2006; Cheng *et al.*, 2007) and molluscs (Lahlou and Berrada, 2001). The biological activities of essential oils have been attributed to the composition or specific essential oil constituent, for example: aldehydes in lemon grass (*Cymbopogon citrates*) have been reported to have anti-inflammatory properties (Boukhatem *et al.*, 2014). Ketones in sweet fennel (*Foeniculum vulgare*) have been found to aid in wound healing and dissolve mucus and fats (Nakatsu *et al.*, 2000). Alcohols in tea tree (*Melaleuca alternifolia*), true lavender (*Lavandula angustifolia*) and baboon wood (*Virola surinamensis*) have anti-microbial and anti-malarial properties (Lopes *et al.*, 1999; Cowan, 1999). Esters in clarry sage (*Salvia sclarea*) have anti-cholinesterase properties (Savalev *et al.*, 2003). Phenols in thyme (*Thymus vulgaris*) and in clove (*Eugenia caryophyllata*) have antimicrobial properties and can be used as food preservatives (Nakatsu *et al.*, 2000). Overall, essential oils are pertinent to pharmaceutical, cosmetic and food research and are widely viewed as templates for structure optimization programs with a goal to creating new drugs (Cragg *et al.*, 1997).



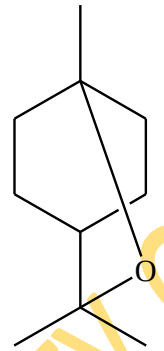
Citronellal **2.29**



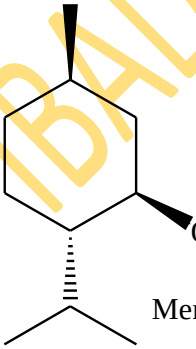
Piperitone **2.30**



Linalyl acetate **2.31**

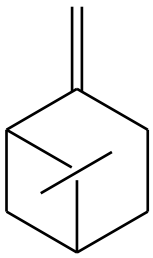


1,8-Cineole **2.32**



Menthol **2.33**

Thymol **2.34**



β-Pinene **2.35**

2.4.1 Extraction of Essential Oils

Essential oils are extracted from different aromatic plants generally distributed in Mediterranean and tropical countries across the world where they are highly regarded as an important component of either native medicine, food or other products (Hussain *et al.*, 2009). These essential oils are accumulated in secretary cells, cavities, channels, and epidermic cells of almost all plant organs such as flowers, buds, stems, leaves, fruits, seeds and roots etc. (Burt, 2004; Chalchat and Ozcan, 2008; Hussain *et al.*, 2008; Anwar *et al.*, 2009a) and can be extracted when plant organs are fresh, partially dehydrated or dried (Ozcan, 2003; Asekun *et al.*, 2007; Hussain *et al.*, 2008).

The extraction of the essential oil depends mainly on the rate of diffusion of the oil through the plant tissues to an exposed surface from where the oil can be removed by a number of processes. There are different methods, depending upon the stability of the oil, for the extraction of the oil from the plant materials. Steam distillation and hydrodistillation are still in use today as the most important processes for obtaining essential oils from the plants (Baker *et al.*, 2000; Kulisic *et al.*, 2004; Masango, 2004; Sokovic and Van Griensven, 2006). Other methods employed for isolation of essential oils include the use of liquid carbon dioxide or microwaves, low or high pressure distillation employing boiling water or hot steam (Bousbia *et al.*, 2009; Donelian *et al.*, 2009). The essential oils obtained by steam distillation or by expression are generally preferred for food and pharmacological applications. Essential oil extraction is different from the extraction of all the other secondary metabolites due to the volatility of the compounds even at room temperature. The following methods are commonly used:

2.4.1.1 Distillation

Distillation is mainly used for obtaining aromatic compounds from plants. There are different processes used but in all of them, steam is generated either in a boiler or in a distillation tank and is allowed to pass through the aromatic material to rupture the oil glands. The steam and essential oil vapours are then cooled in a condenser and the resulting distillate collected. The essential oil will normally float on top of the distilled water component/hydrosol and can easily be separated. The essential oil obtained is filtered and dried in a dessicator over anhydrous sodium sulphate before its storage.

2.4.1.2 Hydro or water distillation

This is the simplest and usually cheapest distillation method. The plant material is immersed in water and boiled. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapours flow through a coil, where they condense back to liquid, which is then collected in the receiving vessel.

In the laboratory hydro distillation is done using a Clevenger-type apparatus, shown in Figure **2.1**. The method is slow and hence time consuming. Furthermore, the prolonged action of hot water can cause hydrolysis of some constituents of the essential oils such as esters. It's also not a suitable method for large scale distillations and for distillation of high saponin rich plant materials.

2.4.1.3 Steam Distillation

The method which is also referred to as wet steam distillation was developed to overcome the drawbacks of hydro distillation. Direct contact of plant material with a hot furnace bottom is thus avoided. The plant material is supported on a perforated grid below which water is boiled. Steam rises through the plant material vaporizing the essential oil with it and is condensed usually in a coil condenser by cooling water. The method is suitable for distilling leafy materials but does not work well for woods, roots and seeds. The distillation units are cheap, easy to operate and are extremely popular with essential oil producers in developing countries. The method, however, is time consuming, gives poor quality oil yields and oil separation is not complete.

Figure 2.1: Clevenger-type apparatus for Hydrodistillation

2.4.1.4 Direct steam distillation

The boiling point of most essential oil components exceeds that of water and generally lies between 150- 300 ^{o}C. However, in the presence of steam they are volatilised at a temperature close to 100 ^{o}C. The principle behind steam distillation is that two immiscible liquids, when mixed, each exerts a vapour pressure, as if each liquid were pure (Houghton and Raman, 1998). The total vapour pressure of the boiling mixture is therefore equal to the sum of the partial pressures exerted by each of the individual components of the mixture. When the total vapour pressure reaches atmospheric pressure, the mixture starts to boil. The plant material is placed in a still and steam prepared in a separate chamber is forced over it. The temperature of the steam is carefully controlled so as not to burn the plant material or the essential oil. The rate of distillation and yield of the oil are high and the oil obtained is of good quality. However, partial loss of more polar constituents of the oil, due to their affinity for water, may occur (Baker *et al.*, 2000; Masango, 2004). The method is quite expensive and only bigger producers can afford to own the distillation unit, hence it is much popular for the isolation of essential oils on commercial scale (Masango, 2004).

2.4.1.5 Hydro diffusion

Hydro diffusion is a type of steam distillation where steam is fed in from the top onto the botanical material. The process uses the principle of osmotic pressure to diffuse oil from the oil glands. The system is connected and low pressure steam is passed into the plant material from a boiler from the top. The oil and water are collected below the condenser in a typical oil separator. The various components of the essential oils are liberated based on their solubility in the boiling water rather than the order of their boiling points (Srivastava, 1991). The main advantage of this method over steam distillation is that less steam is used hence a shorter processing time and therefore higher oil yield.

2.4.1.6 Liquid Carbon Dioxide Extraction Method

Extraction is carried out in a specially designed high-pressure soxhlet apparatus with supercritical/liquid carbon dioxide (CO_2) as the extracting solvent. The plant materials are put into the extraction columns, which are under high pressure (55–58 bar) and the liquid CO_2 flows through the extraction columns until it is saturated with essential oil. At the end of the extraction, the column is taken and the liquid CO_2 is drained from it.

Extraction is carried out at a low temperature and this allows for maximum preservation of all healthful substances in the extract like the aroma, taste, vitamins and enzymes. The essential oils obtained by this method have been found to be superior in quality and flavour as compared with the conventional steam distilled essential oils (Wood *et al.,* 2006). However, the method is expensive in terms of plant and, in some cases, results in an unusual balance of extracted oil components.

2.4.1.7 Expression

Expression is a method of fragrance extraction where raw materials are pressed, squeezed or compressed and the oils are collected. The method is suitable for plant material with naturally high oil content and is often applied to peels of fruits in the citrus family. There is no heat which may decompose the aromatic compounds and hence damage the oil. Essential oils obtained by this method have superior natural fragrance characteristics. Expression can also be done by machine abrasion where a machine strips off the outer peel of the citrus fruit and the peel is carried in a stream of water into a centrifugal separator where the essential oil is separated from other components. Although the centrifugal separation is done extremely fast, the essential oil is combined with other cell contents for some time and some alteration may occur in the oil due to enzymatic action (Schmeiser *et al.,* 2001).

2.4.1.8 Solvent Extraction

Most flowers contain too little volatile oil to undergo expression and their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvent are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil soluble) plant material.

Although highly fragrant, concretes contain large quantities of non-fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, which is more polar in nature, is used to extract the fragrant oil from the concrete. The alcohol is removed by evaporation, leaving behind the absolute.

Supercritical carbon dioxide is used as a solvent in supercritical fluid extraction. This method has many benefits including avoiding petrochemical residues in the product and the loss of some "top notes" when steam distillation is used. It does not yield an

absolute directly. The supercritical carbon dioxide will extract both the waxes and the essential oils that make up the concrete. Subsequent processing with liquid carbon dioxide, achieved in the same extractor by merely lowering the extraction temperature, will separate the waxes from the essential oils. This lower temperature process prevents the decomposition and denaturing of compounds. When the extraction is complete, the pressure is reduced to ambient and the carbon dioxide reverts to a gas, leaving no residue.

2.4.1.9 Florasols Extraction

This method of extraction uses a new family of benign non-CFC (Chlorinated Fluorocarbons) gaseous solvents known as "Florasol". Florasol is a refrigerant and it was developed to replace Freon. Florasol is an ozone friendly product and it causes no danger to the environment. The advantage is that the extraction of essential oils occurs at or below room temperature so any degradation through temperature extremes does not occur. The only thing that is extracted from the plants is the essential oils. The essential oils are absolutely pure and contain no foreign substances at all. The oils are refered to as phytols thus this method is also refered to as "phytonic process" (Okwudiri, 2015).

2.4.2 Analysis of Essential Oils

The characterisation of essential oil on the basis of their chemical profiles is of great importance due to their multiple applications in different fields of man's day to day activities including pharmacy, perfumery, cosmetics, aromatherapy, and food and beverages industry. The fact that essential oils are complex mixtures of biologically active substances (Morris *et al.*, 1979) proves a real challenge for determining their accurate compositional data. The rapid advances in spectroscopic and chromatographic techniques have totally changed the picture of chemical study of essential oils. Many techniques like IR-spectroscopy, UV-spectroscopy, NMR spectroscopy and gas chromatography have been used for studying the chemical profiles of volatile oils (Bakkali *et al*., 2008; Hussain *et al*., 2008). The increasing importance of essential oils in various domains of human activities has prompted an extensive need of reliable methods for analyses of essential oils.

Literatures on the characterization of essential oils have revealed capillary gas chromatography (GC) with flame ionisation detection (FID), are, in most cases, the

method of choice for quantitative determinations. Many researchers make use of mass spectrometers (MS), coupled with GC, to determine the identities of components (Salzer, 1977; Wilkins and Madsen, 1991; Daferera *et al*., 2000; Juliano *et al*., 2000; Jerkovic *et al*., 2001; Delaquis *et al*., 2002; Hussain *et al.*, 2008; Burt, 2004; Anwar *et al*., 2009a,b). Gas chromatography has been proved to be an efficient method for the characterization of essential oils (Bakkali *et al*., 2008; Anwar *et al*., 2009b). The combination of gas chromatography and mass spectrometry (GC-MS) allows rapid and reliable identification of essential oils components (Yadegarinia *et al*., 2006; Gulluce *et al*., 2007; Anwar *et al*., 2009a). Time-of-flight mass spectrometric (TOF-MS) detection has been increasingly used as a qualitative tool, for the detection of volatile components (Adahchour *et al.*, 2003). Capillary columns selected, in most cases, are HP-5ms, DB-5 (cross-linked 5% diphenyl/95% dimethyl siloxane) or DB-1, also known as SE-30, (polydimethyl siloxane) stationary phases. These more non-polar stationary phases are often complimented by the use of a more polar stationary phase, such as polyethylene glycol (Cavaleiro *et al.*, 2004).

2.4.3 Identification of Essential Oil Components

2.4.3.1 Retention Time (t_R)

Retention time is the time which elapses between sample injection and recording of the peak maximum at constant operational conditions which include oven temperature, carrier gas flow rate and sometimes sample size. Nature of the stationary phase, column length and film thickness of the stationary phase are other factors affecting retention time. Retention time of a solute varies with temperature and flow rate. Maintaining constant conditions throughout an experiment are almost imposible, therefore, it is not always possible to reproduce the retention time for a solute (Robert, 1993). Retention time is given by the following equation:

$$t_R = \left(\frac{1+k}{u}\right) L$$

Where k = capacity factor; L = coumn length; u = true linear gas velocity in the column (Sandra and Bicchi, 1987).

2.4.3.2 Retention Indices (RI)

The retention of any substance is defined as equal to hundred (100) times the carbon number of a hypothetical n-alkane which would have the same adjusted retention time as the substance of interest (Poole and Poole, 1991). The use of retention indices in conjunction with GC/MS studies is well established and many analysts use such procedures in their routine analysis to confirm the identity of unknown components (Jirovetz *et al.,* 2000). Nothwithstanding the wide use of linear retention indices, there must be a note of caution when using such indices in an absolute sense. Data from one laboratory to another will invariably not be exactly reproduced, however, the importance is that combined with mass spectral results, retention data still provide an excellent guide to possible identities of components (Marriott *et al.*, 2001).

2.5 Isolation of Secondary Metabolites by Chromatographic Techniques

Chromatography is a physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction (Tom *et al.*, 2004). It is based on the concept of partition coefficient. The basic principle is that components in a mixture have different tendencies to absorb onto a surface or dissolve in a solvent. The choice of the technique depends largely on the nature of the sample component (solubility and volatility). The chromatographic techniques used in the course of this study are: thin layer chromatography (TLC), column chromatography, gas chromatography (GC) and high performance liquid chromatography (HPLC).

2.5.1 Thin Layer Chromatography

Thin layer chromatography (TLC) is a form of planar chromatography (solid-liquid partitioning mechanism) which is widely employed in the laboratory and similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations and the choice between different adsorbents.

In thin layer chromatography, diluted samples are spotted with the aid of a small diameter capillary micropipette on an evenly spaced spots at about 1 cm from the base of a preparative or analytical TLC plate. Spotted plate is allowed to dry, and then developed in a development chamber containing appropriate solvent system. After development, the separated bands can be visualised possibly by UV light.

Visualisation method can be a qualitative and quantitative technique used in the location of separated component of a crude extract. The methods are tailored to functional groups of metabolites. Metabolites with some level of unsaturation can be located with iodine crystals to give either yellow or brown colouration. Method like UV light for compounds that fluoresce is not destructive, but chemical methods involving the use of reagents like silver halides for detecting alkyl halides, charring of organic components with concentrated H_2SO_4 (tetraoxosulphate (VI) acid), 2,4-DPH (2,4-Dinitrophenyl hydrazine) for carbonyl compounds, ferric chloride for phenolics, ninhydrin for amino acids and p-dimethyl aminobenzaldehyde for amines are destructive (Pavia *et al.*, 2014).

High performance thin layer chromatography (HPTLC) is an improved version of thin layer chromatography (Braithwaite and smith, 1999).

2.5.2 Column Chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. It is an effective method of separating the components of crude extracts which depends on: the length and diameter of the column, flow rate, nature of adsorbent and mobile phase employed. The separating technique works on the net distribution of the components of a mixture between the adsorbent and the mobile phase, owing to selective adsorptivity and solubility (Pavia *et al.*, 2014).

Pre-adsorbed material is loaded on a packed column, elution is achieved isocratically or gradiently and the column is monitored using microscope slide TLC. Fractions are collected, distilled and identified with respective R_f values. Isolates with the same R_f values are combined and purity check is carried out. Differences in rates of movement through the medium are calculated to different retention times of the sample (Ettre, 1993).

2.5.3 Gas Chromatography

Gas chromatography (GC) also known as gas-liquid chromatography (GLC) is one of the most useful tools for separating and analysing organic compounds that can be vaporised without decomposition. It resembles column chromatography in principle with technique in which the mobile phase is a gas. The partitioning processes for mixtures are carried out between an inert moving gas phase and a stationary liquid phase, at a controllable temperature. The concentration of any compound in the gas

phase is a function of its vapour pressure; which is the basis of isolation (Pavia *et al.*, 2014).

Gas chromatography has a wide range of application. These include drugs and consumer products analysis, environmental monitoring of air, water and legislation. Gas chromatography-mass spectrometry (GC-MS) is advanced gas chromatography technique which uses mass spectroscopic detectors. This allows sample mixtures containing common organic analyte to be separated and identified using a single bench-top instrument.

Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) is a technique for the analysis and quantitation of organic volatile and semi-volatile compounds. The advantage of the coupling of a chromatographic device to a spectrometer is that complex mixtures can be analyzed in detail by spectral interpretation of the separated individual components. The coupling of a gas chromatograph with a mass spectrometer is the most often used and a well established technique for the analysis of essential oils, due to the development of easy-to-handle powerful systems concerning sensitivity, data acquisition and processing, and above all their relatively low cost.

Majority of GC-MS applications utilize one-dimensional capillary GC with quadrupole MS detection and electron ionization. Nevertheless, there are substantial numbers of applications using different types of mass spectrometers and ionization techniques. The proliferation of GC-MS applications is also a result of commercially available easy-to-handle dedicated mass spectral libraries like NIST/EPA/NIH 2005; WILEY Registry 2006; MassFinder 2007; NIST 2011 and diverse printed versions such as Jennings and Shibamoto, 1980; Joulain and Koenig, 1998 and Adams, 1989, 1995, 2007 inclusive of retention indices providing identification of the separated compounds. However, this type of identification has the potential of producing some unreliable results, if no additional information is used, since some compounds, for example, the sesquiterpene hydrocarbons α-cuprenene and β-himachalene, exhibit identical fragmentation pattern and only very small differences of their retention index values. This example demonstrates impressively that even a good library match and the additional use of retention data may lead in some cases to questionable results, and therefore require additional analytical data, for example, from NMR measurements.

2.5.4 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) also known as High Pressure Liquid Chromatography is a modification of the column chromatography with the aim of achieving better isolations with tightly packed columns under pressure. Thus, the solvent flow rate is increased and a better resolution is achieved. HPLC is operated at ambient temperature and the compounds are therefore not subjected to thermal rearrangement during the separation. HPLC is mainly used for some classes of compounds which are non-volatile e.g. higher terpenoids, phenolics, alkaloids, lipids and sugars (Harbone, 1998).

2.6 Spectroscopic Techniques

Pure isolates are often subjected to structural investigation so as to determine their structures using combined spectral techniques of modern methods of spectroscopic studies. Spectroscopic techniques all work on the principle of absorbance or emission of energy. The methods have been established as an authentic as well as one of the most significant techniques not only in solving structural problems but also in analytical and preparative works (Brahmachari, 2009). The methods used in this study include: Ultraviolet-Visible, Infra-Red, Nuclear Magnetic Resonance and Mass Spectrometry.

2.6.1 Ultraviolet (UV) – Visible Spectroscopy

The presence of conjugated system in an organic compound can be determined by UV-visible spectroscopy, since they will be absorbed in the UV-visible region (200-400 nm) and then experience a transition in the electronic level, thus, yielding characteristic absorption bands. The transition (excitation) is usually from the ground state to higher energy state. The energy of the ultraviolet radiation absorbed is equal to the energy difference between the ground state and higher energy states ($\Delta E = hf$). Generally, the most favoured transition is from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) (Pavia *et al.*, 2001; Kalsi, 2004).

UV employs the Beer-Lambert's law which explains that the greater the number of molecule capable of absorbing light of a given wavelength, the greater the extent of light absorption. Furthermore, the more effectively a molecule absorbs light of a given

wavelength, the greater the extent of light absorption. Beer-Lambert's law relates absorption to the concentration of absorbing solute (log (I_O/I) = Ɛcl). It also relates the total absorption to optical path length (Ɛ = A/cl).

The effect of solvents and substitution cannot be overemphasised. This could however, bring about a shift in absorption maximum (λ_{max}) or intensity of absorption (E_{max}). The information obtained helps to deduce the presence of chromophoric systems: ethylenic (isolated or conjugated), acetylenic unsaturations, carbonyls, acids, esters, nitro and nitrile groups in the metabolite under investigation (Brahmachari, 2009).

2.6.2 Infra-red Spectroscopy

Infra red (IR) spectroscopy is a method of characterisation which gives sufficient information on the structural pattern of organic compounds, in the region 4000- 400 cm^{-1}. As IR radiation is passed through a sample, specific wavelengths are absorbed causing the chemical bonds in the material to undergo vibrations such as stretching, contracting and bending (Tolstoy *et al.*, 2003). Organic compounds containing functional groups that absorb in the infra red region of the electromagnetic spectrum are readily determined either in the neat or concentrated or diluted form. Factors like hydrogen bonding, electronic effect, field effect, ring strain, atomic mass and vibrational coupling often influence the relative absorption of organic functionalities (Brahmachari, 2009).

2.6.3 Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) Spectroscopy is a sophisticated technique often employed in the study of molecules by means of interaction of nuclei of these molecules, placed in a strong magnetic field, with radiofrequency electromagnetic radiation. NMR can provide information about the structure, dynamics, reaction state and chemical environment of molecules.

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. On application of an external magnetic field, an energy transfer is possible between the base energy to a higher energy level. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed to yield an NMR spectrum for the nucleus

concerned (Pavia *et al.*, 2014; Kalsi, 2004). Common NMR active nuclei are ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P and ^{29}Si. One dimensional NMR involves acquiring data as a function of one time variable while two dimensional NMR involves acquiring data as a function of two time dependent variables.

Distortionless Enhancement by Polarisation Transfer (DEPT) and Attached Proton Transfer (APT) are examples of a 1-D NMR (Das and Mahato, 1983; Mahato *et al.*, 1992). The three types of DEPT are: DEPT 45, 90 and 135. Two dimensional NMR includes: J-Resolved e.g. COSY (Correlation Spectroscopy), TOCSY (Total Correlation Spectroscopy) or NOESY (Nuclear Overhauser Effect Spectroscopy) and Correlation experiments e.g. HETCOR (Heteronuclear Correlation Spectroscopy), HSQC (Heteronuclear Single Quantum Correlation), HMQC (Heteronuclear Multiple Quantum Correlation) or HMBC (Heteronuclear Multiple Bond Correlation). Two dimensional NMR generally provides information about bonds connectivity (Williams and King, 1990).

2.6.3.1 ^{1}H NMR Spectroscopy

Proton NMR spectroscopy essentially provides means of determining the structure of an organic compound by measuring the magnetic moments of its hydrogen atoms. In most natural products, hydrogen atoms are attached to different groups and the proton NMR spectrum provides information about the number of hydrogen atoms in these different environments.

2.6.3.2 ^{13}C NMR Spectroscopy

^{13}C experiment allows for the identification of carbon atoms in organic compounds. As such ^{13}C NMR detects only ^{13}C isotope of carbon, whose abundance is only 1.1%, because the main carbon isotope, ^{12}C is not detectable by NMR. ^{13}C NMR has a number of complications that are not encountered in ^{1}H NMR. (Caytan *et al.*, 2007).

2.6.3.3 Distortionless Enhancement by Polarization Transfer (DEPT)

It is a very useful method for determining the presence of primary, secondary and tertiary carbon atoms. The DEPT experiment differentiates between CH, CH_2 and CH_3 groups by variation of the selection angle parameter. DEPT 45 gives all carbons with attached protons (regardless of number) in phase. DEPT 90 gives only CH groups, the

others being suppressed. DEPT 135 indicates CH and CH_3 in positive mode while CH_2 is in negative mode (Caytan *et al.*, 2007).

2.6.3.4 ^{I}H-^{1}H Correlation Spectroscopy (COSY)

Homonuclear correlation spectroscopy shows correlation between protons that are coupled to each other. It is the first and most popular two-dimension NMR which is used to identify spins which are coupled to each other. In ^{I}H-^{I}H correlation spectroscopy, coupling usually occur over two or three bonds (germinal or vicinal coupling respectively) and its presence provides direct evidence of a bond within a structure; it indicates connectivity. If the connectivity between all atoms in a structure is known, the gross structure is, therefore, defined.

The two-dimensional spectrum that results from the COSY experiment shows the frequencies for a single isotope, most commonly hydrogen (^{1}H) along both axes. COSY spectra shows two types of peaks namely the diagonal peak and cross peak. Diagonal peaks have the same frequency coordinate on each axis and appear along the diagonal of the plot; while cross peaks have different values for each frequency coordinate and appear off the diagonal. Diagonal peaks correspond to the peaks in a 1D-NMR experiment, while the cross peaks indicate couplings between pairs of nuclei (Keeler, 2010).

2.6.3.5 Heteronuclear Single Quantum Coherence (HSQC)

This is a C-H correlation experiment which uses proton detection of the ^{13}C signals. HSQC detects correlations between nuclei of two different types which are separated by one bond. This method gives one peak per pair of coupled nuclei, whose two coordinates are the chemical shifts of the two coupled atoms. Heteronuclear multiple-quantum correlation spectroscopy (HMQC) gives an identical spectrum as HSQC, but using a different method. The two methods give similar quality results for small to medium sized molecules, but HSQC is considered to be superior for larger molecules (Schram and Bellama, 1988).

2.6.3.6 Heteronuclear Multiple Bond Correlation

Heteronuclear Multiple Bond Correlation (HMBC) is closely related to HMQC and operates in essentially the same manner. In this case, however, the correlation is across more than one bond that arises from so-called long-range couplings (J=2-15 Hz). Cross

peaks are between protons and carbons that are two or three bonds away (and sometimes up to four or five bonds away in conjugated systems). Direct one bond cross peaks are suppressed. This experiment is analogous to the proton–proton COSY experiment in that it provides connectivity information over several bonds.

2.6.3.7 Nuclear Overhauser Effect Spectroscopy (NOESY)

These methods establish correlations between nuclei which are physically close to each other regardless of whether there is a bond between them. In NOESY, the Nuclear Overhauser cross relaxation between nuclear spins during the mixing period is used to establish the correlations. The spectrum obtained is similar to COSY, with diagonal peaks and cross peaks, however the cross peaks connect resonances from nuclei that are spatially close rather than those that are through-bond coupled to each other. This method is a very useful tool to study the conformation of molecules and for determining the 3-dimensional structure of molecules (Lamber and Mazzola, 2002)

2.6.4 Mass Spectrometry

Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information and can be conducted on tiny samples (Pavia *et al.*, 2014; Kalsi, 2004). Therefore, mass spectroscopy allows quantitation of atoms or molecules and provides structural information by the identification of distinctive fragmentation patterns. The instrument used in mass spectrometry analysis is mass spectrometer. The mass spectrometer operation can be divided to three part namely, creation of gas-phase ions, separation of the ions on their mass-to-charge ratio and measurement of the quantity of ions of each mass-to-charge ratio. These three phases of operation are carried out by suitable ionisation source, mass analysers and detector respectively.

2.6.4.1 The Ionisation source

Ionisation source converts gas phase sample molecules into ions. Examples include Chemical Ionisation (CI), Electron Impact (EI), Electrospray Ionization (ESI), Fast Atom Bombardment (FAB), Field Desorption/Field Ionisation (FD/FI), Matrix

Assisted Laser Desorption Ionisation (MALDI) and Thermospray Ionisation (TI) (Kalsi, 2004).

2.6.4.2 Electron Impact Ionisation

This is obtained by passing a beam of electrons through a gas-phase sample and collides with neutral analyte molecules (M) to produce a positively charged ion or a fragment ion. This method is applicable to all volatile compounds and gives reproducible mass spectra with fragmentation to provide structural information (Kalsi, 2004).

2.6.4.3 Electrospray Ionization (ESI)

Electrospray Ionisation is obtained by nebulizing solution under atmospheric pressure and exposed to a high electrical field which creates a charge on the surface of the droplet. The production of multiple charged ions makes electrospray extremely useful for precise mass measurement (Kalsi, 2004).

2.6.4.4 Fast Atom Bombardment (FAB)

Fast Atom bombardment method of ionisation generates ion by using a high current of bombarding particle to bombard the analyte which is in low volatile liquid matrix. This is a soft ionisation technique and is suitable for analysis of low volatility species (Kalsi, 2004).

2.6.4.5 Chemical Ionisation

Chemical ionisation method employed the ionisation of a reagent gas by electron impact and then subsequently reacts with analyte molecules to produce analyte ions. This method gives molecular weight information and reduced fragmentation in comparison to EI (Kalsi, 2004).

2.6.4.6 The mass analyzer

Mass analyser sorts ions by their masses by applying electromagnetic fields. Examples include quadrupoles, Time-of-Flight (TOF), magnetic sectors, fourier transform and quadrupole ion traps (Kalsi, 2004).

2.7 Biological Activities of Essential Oils

The biological activities of essential oils are virtually the basis for which the importance of the oils is determined. The bioactivity of the oils can be compared with the activity of synthetically produced pharmacological preparations and investigated in the same way ensuring that the factors that can affect the exactness of the activity are put into consideration. The activity of the essential oils is related to composition, functional groups and synergistic interactions between components (Dorman and Deans, 2000).

2.7.1 Insecticidal Activity

Although stored grains can be destroyed by insects, fungi, and vertebrate pests, insect pests are often the most important because of the favorable environmental conditions that promote their development. Freedom from insect infestation and contamination has become an important consideration in storage of grain and to maintain a high quality product (Collins, 1998). Nearly one thousand species of insects have been associated with stored products throughout the world, of which the majority belong to Coleoptera (60%) and Lepidoptera (8-9%) (Schwartz and Burkholder, 1991; Kucerova *et al.*, 2003). Fumigants are mostly used against stored-grain insect pests, not only because of their broad activity spectrum, but also because of their penetrating power resulting in minimal or no residues on the treated products. Although effective fumigants (e.g. methyl bromide and phosphine) are available, there is global concern about their negative effects, such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance, and pesticide residues (Hansen and Jensen, 2002; Benhalima *et al.*, 2004; Bughio and Wilkins, 2004). Thus, there is an urgent need to develop safe alternative fumigants for stored-grain pest management. Herbal products are one potentially important source.

A large number of plant essential oils have been used against diverse insect pests since they, unlike conventional pesticides, present no risk to humans and the environment (Soares *et al.*, 2008; Lima *et al.*, 2009, 2011).

Sahaf *et al*. (2007) found a strong insecticidal activity of the *Carum copticum* C.B. Clarke (Apiaceae) essential oil on *Sitophilus oryzae* (L.) (Curculionidae) and *T. castaneum*. In another experiment, Chaubey (2008) studied the fumigant activity of *Anethum graveolens* L. and *Cuminum cyminum* L. essential oils on *Callosobruchus*

chinensis (L.) (Bruchidae). Lopez *et al*. (2008) reported that *Carum carvi* L. and *Coriandrum sativum* L. were toxic against *Rhyzopertha dominica* (F.) (Bostrichidae) and *S. oryzae*.

Commonly used bioassay techniques for the determination of insecticidal activity are Insecticide impregnated dust on grain (Champ, 1981), Direct spray on grain (Champ, 1981), Impregnated filter paper test (Tabassum *et al.,* 1997) and Toxicity on various surfaces (Atta-ur-Rahman *et al.,* 2001)

2.7.1.1 Common Stored Grain Pests

***Tribolium castaneum* (Red Flour Beetle)**

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: *Tenebrionidae*), the darkling beetles, can be a major pest in stored grains. This species has been found associated with a wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits, and spices, but milled grain products such as flour appear to be their preferred food (Campbell and Runnion, 2003). It is a worldwide pest of stored products, particularly food grains, and a model organism for ethological and food safety research (Grünwald, 2013). It may cause an allergic response, but is not known to spread disease or cause damage to structures or furniture. The United Nations, in a recent post-harvest compendium, estimated that *Tribolium castaneum* and *Tribolium confusum*, the confused flour beetle, are "the two most common secondary pests of all plant commodities in store throughout the world (Sallam, 2008).

The red flour beetle is of Indo-Australian origin and less able to survive outdoors than the closely related species *Tribolium confusum*. It has, as a consequence, a more southern distribution, though both species are worldwide in heated environments. The adult is long-lived, sometimes living more than three years. Although previously regarded as a relatively sedentary insect, it has been shown in molecular and ecological research to disperse considerable distances by flight (Ridley *et al.,* 2011).

Female red flour beetles are polyandrous in mating behaviour in order to increase their fertility assurance. Within a single copulation period, a single female will mate with multiple different males. By mating with an increased number of males, female beetles obtain a greater amount of sperm since many sexually active male red flour beetles are non-virgins and may be sperm depleted. It is important to note that red flour beetles

engage in polyandry to obtain a greater amount of sperm from males, not to increase the likelihood of finding genetically compatible sperm (Pai *et al.*, 2005).

***Rhyzopertha dominica* (Lesser grain borer)**

Rhyzopertha is a monotypic genus of beetles in the family *Bostrichidae*, the false powderpost beetles. The sole species, *Rhyzopertha dominica*, is known commonly as the lesser grain borer, American wheat weevil, Australian wheat weevil, and stored grain borer. It is a beetle known nearly worldwide as a pest of stored cereal grains (Granousky, 1997).

***Callosobruchus analis* (Pulse beetle)**

Callosobruchus is a genus of beetles in the family Coleoptera: *Chrysomelidae*, the leaf beetles. It is in the subfamily *Bruchinae*, the bean weevils (Tuda *et al.*, 2006). Many beetles in the genus are well known as economically important pests that infest stored foodstuffs (Tuda *et al.*, 2006). These beetles specialize on legumes of the tribe *Phaseoleae*, which includes many types of beans used for food. Host plants include mung bean (*Vigna radiata*), adzuki bean (*V. angularis*), rice bean (*V. umbellata*), cowpea (*Vigna unguiculata*), Bambara groundnut (*V. subterranea*), pigeon pea (*Cajanus cajan*), lablab (*Lablab purpureus*), and common bean (*Phaseolus vulgaris*) (Tuda *et al.*, 2006). They can also be found in peas, lentils, chickpeas, and peanuts (Tuda *et al.*, 2005). Most species in the genus are native to Asia. They can be found in warm regions in the Old World. They occur in places outside of their native range as introduced species. At least 11 species of legumes are natural hosts for these beetles, including wild and domesticated plants. Some are considered pests because they invade stores of legume foods, such as beans and lentils. They lay eggs on the seeds and the larvae consume them as they develop. They emerge from the seeds as adults (Tuda *et al.*, 2005).

Callosobruchus spp. cause a potential loss in legume by feeding on the protein content of the grain and their damage ranges from 12-30% in developing countries (Tsedeke, 1985; FAO, 1994). Field infestation by *Callosobruchus* spp. appears to be very common (Mohan and Subbarao, 2000; Messina, 1984). This field infestation though occurs at a very low level, acts as a potential source of initiation of population buildup during post-harvest period in stores causing heavy losses (Khavilkar and Dalvi, 1984).

Table 2.3: Pictures of Insects Used for the Study

Insect Biological Name	Common Name	Picture
Tribolium castaneum	Red Flour Beetle	 
Rhyzopertha dominica	Lesser grain borer	
Callosobruchus analis	Pulse beetle	

2.7.2 Antibacterial Activity

Antibiotic resistance of organisms is a global threat (Zinn *et al.*, 2004). There is a need to search for new compounds (that are not penicillin based) that inhibit microbial growth. Essential oils are known to exhibit antimicrobial properties that are lethal or static to the growth of bacteria, fungi, or virus (Oka *et al.*, 2000; Janssen *et al.*, 1987). They are used in the prevention and treatment of infections, with respect to their preservative and antimicrobial properties, in food products, in cosmetics and as disinfectants (Palevitch, 1994; Suppakul *et al.*, 2003a,b). Seenivasan *et al.* (2006) while working on the *in vitro* antibacterial activity of six plant essential oils observed that cinnamon clove and lime oils inhibited antibacterial activity on both gram-positive and gram-negative bacteria. The minimum inhibitory and bactericidal concentration values were reported for the essential oils of *Eucalyptus globulus* as well as that of *Thymus algeriensis*, suggests that these oils have the potential to be used as natural agents in preservatives for food and pharmaceutical products (Abdenour *et al.*, 2011). The essential oil of *Blumea megacephala* is a newly discovered potential source of natural antimicrobial compounds (Zhu *et al.*, 2011). The study of Lalitha *et al.*, (2011) confirms that many essential oils as well as plant extracts possess *in vitro* antifungal and antibacterial activity. The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms has been reported (Lis-Balchin *et al.*, 1998; Rojas-Grau *et al.*, 2007).

In general, the higher antimicrobial activity of essential oils is observed on gram-positive bacteria than gram-negative bacteria (Kokoska *et al.*, 2002; Okoh *et al.*, 2010). Lipophilic ends of lipoteichoic acids in cell membrane of gram positive bacteria may facilitate the penetration of hydrophobic compounds of essential oils (Cox *et al.*, 2000). On the other hand, the resistance of gram-negative bacteria to essential oils is associated with the protecting role of extrinsic membrane proteins or cell wall lipopolysaccharides, which limits the diffusion rate of hydrophobic compounds

through the lipopolysaccharide layer (Burt, 2004). The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death (Ultee *et al.,* 1999). The cytoplasmic membrane of bacteria generally has two (2) principal functions:

(i) barrier function and energy transduction, which allow the membrane to form ion gradients that can be used to drive various processes, and

(ii) formation of a matrix for membrane-embedded proteins (such as the membrane-integrated complex of ATP synthase) (Sikkema *et al.,* 1995; Hensel *et al.,* 1996).

The activity of the essential oils is related to composition, functional groups, and synergistic interactions between components (Dorman and Deans 2000).

Plant essential oils have been known as antimicrobial agents. Essential oil of rosemary (*R. officinalis*) exhibited good activity against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria (Okoh *et al.,* 2010). The major components of rosemary oil are monoterpenes such as α-pinene, β-pinene, myrcene 1,8-cineole, borneol, camphor, and verbinone (Santoyo *et al.,* 2005; Okoh *et al.,* 2010), which possess strong antimicrobial activity by the disruption of bacteria membrane integrity (Knobloch *et al.,* 1989).

Aguirre *et al.,* (2013); Burt (2004) and Pelissari *et al.,* (2009) also reported that oregano essential oil had higher antimicrobial activity against the gram-positive bacteria (*S. aureus*) than gram-negative (*E. coli* and *Pseudomonas aeruginosa*). The main constituents of oregano essential oil are thymol, carvacrol, γ-therpinene and ρ-cymene (Lambert *et al.,* 2001; Burt 2004; Aguirre *et al.,* 2013). However, *Pseudomonas putida* was resistant to carrot seed and parsley essential oils (Teixeira *et al.,* 2013).

E. coli and *Salmonella typhimurium* were also tolerant to carrot seed, grapefruit, lemon, onion, and parsley essential oils. The greater resistance of gram-negative bacteria toward essential oils may be attributed to the complexity of their double-layer cell membrane, compared with the single-layer membrane of gram-positive bacteria (Hogg, 2005).

Antimicrobial activity of *Callistemon comboynensis* essential oil was observed against gram-positive (*B. subtilis* and *S. aureus*), gram-negative (*Proteus vulgaris* and *P. aeruginosa*), and a pathogenic fungus *Candida albicans*. This might be associated with the high content of oxygenated constituents; 1,8-cineole (53.03%), eugenol (12.1%), methyl eugenol (8.3%), α-terpineol (4.3%), and carveol (3.4%) (Abdelhady and Aly, 2012). Teixeira *et al.*, (2013) found that the highest reduction was obtained when coriander, origanum and rosemary essential oils at a level of 20 µL were used to inhibit *Listeria innocua*. Thyme essential oil (20 µL) was able to inhibit both *L. innocua* and *Listeria monocytogenes*. Thus, essential oils from the selected plants can be used as antimicrobial agents for food applications as well as other purposes. However, their activity depends on types of essential oil used.

Different assays like the disc diffusion assay, well diffusion assay, micro dilution assay, measurement of minimum inhibitory concentration and microplate alamar blue assay are often used for measuring the antimicrobial activity of essential oils and plant based constituents (Bakkali *et al.*, 2008). However, factors such as volume of the inoculums, growth phase, culture medium used, pH of the media, incubation time and temperature have made comparison of published data complicated (Viljoen *et al.*, 2003; Sonboli *et al.*, 2006). Despite the differences in the methods of assessment, it is apparent that many plant species contain anti-microbial compounds. A relationship between chemical structure of the volatile and non-volatile plant constituents and antimicrobial activity has been reported (Farag *et al.*, 1989). Examples of antibacterial compounds in essential oils include 1,8-cineole **(2.32)**, linalool, thymol **(2.34)**, eugenol, carvacrol, α-pinene, menthol **(2.33)**, β-pinene **(2.35)** (Kalemba and Kunicka, 2003; Firas, 2009).

2.7.2.1 Properties of Selected Bacteria Species

Staphylococcus aureus (Gram-positive) is a major cause of hospital-acquired infections, pneumonia, and staphylococcal meningitis and it has been reported to be resistant to ciprofloxacin, erythromycin, clindamycin, gentamicin, trimethoprim /sulphamethoxazole, linezolid and vancomycin (Styers *et al.*, 2006).

Pseudomonas aeruginosa (Gram-negative), which is a major cause of infectious-related mortality among the critically ill patients, is resistant to a large number of

antibiotics (Bonfiglio *et al.*, 1998; Gales *et al.*, 2001) and hence carries the highest case fatality rate of all Gram negative infections (Aliaga *et al.*, 2002).

Escherichia coli (Gram-negative) also cause life threatening infections especially in the hospital environment and has been found to be resistant to commonly used antimicrobial agents *like* trimethoprim-sulfame-thoxazole (Amyes and May, 2007).

Shigella flexineri (Gram-negative) is a human intestinal pathogen, causing dysentery by invading the epithelium of the colon and is responsible, worldwide, for an estimated 165 million episodes of shigellosis and 1.5 million deaths per year (Nanyonga, 2012). Resistance of *Shigella flexineri* to antimicrobial agents has also been reported (Sack *et al.*, 1997).

Salmonella typhi (Gram-negative) is a genus of bacteria that cause typhoid fever which is a major health problem especially in developing countries (Lin *et al.*, 2000; Otegbayo *et al.*, 2003) and multidrug-resistant strains of *Salmonella* have been encountered (Olowe *et al.*, 2003).

Bacillus subtillis are Gram positive large rods that are widely spread in air soil and water (Oyedeji, 2001). It causes food poisoning.

2.7.3 Antioxidant Activity

Human body consumes ample amounts of oxygen for the metabolism of biomolecules in order to produce energy. Although oxygen is essential for life, but its metabolites, so called reactive oxygen species (ROS), are very toxic and cause harm to cells. An imbalance between ROS production and elimination leads to an “oxidative stress”. Antioxidants are uniquely qualified to decrease the oxidative stress and neutralize ROS before they damage the tissues. Hence a variety of antioxidants is required for neutralization of free radicals to protect body from their adverse effects. An antioxidant is thus a substance that, when present at a low concentration compared with that of an oxidisable substrate, inhibits oxidation of the substrate (Halliwell and Gutteridge, 2007).

Chemically, free radicals are molecules that are loosing an electron and this makes them highly reactive as oxidants. In the act of desperately snatching an electron from any other molecule, ROS exert oxidative damaging effects to molecules found in living

cells including DNA (Sharma *et al.*, 2001). Damage to DNA, if not reversed by DNA repair mechanisms, can cause mutations and possibly cancer (Valko *et al.*, 2004). There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, liver injury, atherosclerosis, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases and aging (Davies, 2000; Fenkel and Holbrook, 2000).

Antioxidant activities of essential oils have been studied widely and reported. Guimarães (2010) investigated the antioxidant activity of essential oils of *Lippia sidoides*, *Alomia fastigiata*, *Ocotea odorifera*, *Mikania glauca* and *Cordia verbenacea*, and their majority constituents, by the methods of the β-carotene/linoleic acid oxidation system and the reduction of the stable DPPH radical. The essential oil of *L. sidoides* showed higher antioxidant activity, presenting the lowest IC_{50} values in all trials, and the antioxidant activity presented by the essential oil of *L. sidoides* was attributed to its major constituent, carvacrol, which also showed high antioxidant activity when assessed in isolation. Mothana (2011) working with the essential oils of *Nepetade flersiana* growing in Yemen showed that the oil was able to reduce DPPH and to demonstrate a moderate antioxidant activity although, the observed low antioxidant activity could be associated with low content of phenolic compounds such as thymol and carvacrol in the investigated oil. The essential oils from guava stem bark were seen to be a weak proton donor in DPPH reaction. However, compared favorably with α-tocopherol a good scavenger of hydroxyl radical (Fasola *et al.*, 2011). Kadri *et al.* (2011) while working on the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-arid region postulated that antioxidant activities of the essential oil studied may be a potential source of natural antioxidants in foods in order to find possible alternative to synthetic antioxidant, and the pharmaceutical industry for the prevention and the treatment of various human diseases. Hammami *et al.* (2011) discovered that the essential oil obtained from flowers of *G. sanguineum L.* possessed antibacterial and antioxidant activities.

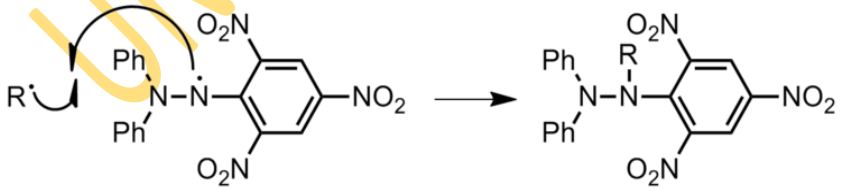
Antioxidant properties of essential oils may make them a very good candidate for use as natural antioxidants and also a model for new free radical scavenging drugs.

The most popular and frequently used for the determination of antioxidant activity of volatile and non volatile plant extracts is the DPPH radical scavenging assay (Hussain *et al.*, 2010).

Related to DPPH, is the 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging assay. Bleaching of β-carotene in linoleic acid system is also a simple, reproducible and time efficient method for rapid evaluation of antioxidant properties and has been employed in many studies for evaluating the antioxidant potential of essential oils and plant extracts (Hussain *et al.*, 2010). Other methods include ferric reducing antioxidant power (FRAP), nitric oxide (NO) radical inhibition, chelating effect of ferrous ions, hydrogen peroxide scavenging activity and superoxide anion scavenging activity (Yen and Chen, 1995).

DPPH Radical Scavenging Assay

This spectrophotometric assay uses the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Yadegarinia *et al.*, 2006). DPPH is a dark-colored crystalline powder composed of stable free-radical molecules and has major application in laboratory research most notably in antioxidant assays. The model of scavenging stable DPPH-free radicals can be used to evaluate the antioxidative activities in a relatively short time (Conforti *et al.*, 2006). The samples are able to reduce the stable free DPPH radical to 2,2-diphenyl-1-picrylhydrazine that is yellow colored. The hydrogen or electron donation abilities of the samples are measured by means of the decrease of the absorbance resulting in a color change from purple to yellow (Gutierrez *et al.*, 2006).



2,2-diphenyl-1-picrylhydrazyl 2,2-diphenyl-1-picrylhydrazine

2.8 Fruit Plant Samples

2.8.1 *Persea americana* Mill (Avocado Pear)

Brief Description

Pear is the name for about twenty (20) species of trees of a genus in the rose family, and for their fruit. *Persea americana* Mill (*Lauraceae*) commonly called Avocado is usually medium sized, erect and deciduous tree ranging from 15-20 m in height (Ojewole *et al.*, 2007) with trunks 30 cm (12 inches) or more in diameter. The leaves are oval and simple and, unlike those of the apple, smooth and glossy. The white flowers, which are borne in umbels, have five sepals, five petals, many stamens, and a single pistil. The fruit is a pome, juicier than the apple, and varying from apple-shaped to teardrop-shaped (Figure **2.2**). Among different varieties, the thin skin varies in color from light yellow and green through red and brown. The thick flesh varies in flavor among different varieties. Pears are gathered from the trees before they are completely ripe and are allowed to ripen in storage. Cold retards ripening, and heat speeds it. Most pear varieties may be grown in either standard or dwarf sizes.

Ethnomedicinal Importance

Different parts of the plant are used in folk medicine for the treatment of several ailments such as hypertension, diabetes and inflammation (Adeyemi *et al.*, 2002; Lans, 2006; Bartholomew *et al*, 2007). Pears are eaten fresh and canned. The fruit contain about 16% carbohydrate and negligible amounts of fat and protein. They are good sources of the B-complex vitamins and also contain vitamin C; in addition, they contain small amounts of phosphorus and iodine. Specifically the fruit is used as vermifuge, for treatment of dysentery and as aphrodisiac (Bartholomew *et al.*, 2007). The leaves are used extensively in the treatment of hypertension (Gill, 1992; Lans 2006), sore throat, haemorrhage and inflammatory conditions (Bartholomew *et al.*, 2007). Some of the scientifically validated activities of the plant leaves include its antihypertensive activity (Owolabi *et al.*, 2005; Ojewole *et al.*, 2007), anticonvulsant effect (Ojewole and Amabeoku, 2006), analgesic and anti-inflammatory activities (Adeyemi *et al.*, 2002).

The leaves have been reported as an effective antitussive and antidiabetic, and for relief of arthritis pain, by traditional medicine practitioners of the Ibibio tribe in South Nigeria. The leaves have been reported to be effective anti-tussive, antidiabetic, and anti-arthritic by traditional medicine practitioners of Ibibio tribe of Southern Nigeria. Analgesic properties of the leaves have also been reported (Anita *et al.*, 2005).

The seeds (crude or toasted) are employed in traditional Mexican medicine to treat skin rashes, diarrhea, and dysentery caused by helminths and amoebas, for the cure of infectious processes caused by fungi and bacteria, as well as for the treatment of asthma, high blood pressure, and rheumatism (Aguilar and Aguilar, 1994; Argueta *et al.,* 1994; Adeyemi *et al.,* 2002; Del-Refugio-Ramos *et al.,* 2004; Osuna-Torres *et al.,* 2005; Moreno-Uribe, 2008; Anaka *et al.,* 2009). The seeds of *P. americana* used alone or mixed with other species, such as *Psidium guajava*, *Mentha piperita* or *Ocimum basilicum*, are mainly employed for the treatment of diarrhea (Osuna-Torres *et al.,* 2005).

Previous Work

Results from previous investigation into the chemical composition of *P. americana* leaf, fruit and seed showed that the investigated samples contain phytochemicals such as phenols, saponins, tannins, steroids, alkaloids and flavonoids (Arukwe *et al.*, 2012). Proximate content revealed that the fruit of *P. americana* contains more of fat and energy; seed had more of fat, protein and energy while the leaf had more of protein, fibre, and ash (Arukwe *et al.*, 2012).

P. americana has been reported to be effective against hepato-toxicity, inflammation, cancer and hypertension (Adeyemi *et al.,* 2002; Anaka *et al.,* 2009; Imafidon and Amaechina, 2010; Ojewole and Amabeoku, 2006).

The presence of fatty acids (linoleic, oleic, palmitic, stearic, linolenic, capric and myristic acids), polyphenols (catechin, isocatechin, protocyanidin, flavonoids, tannins and proanthocyanidin monomerics), saponins, glucosides (D-perseit, D-α-manoheptit, D-monoheptulose, persiteol), sterols (β-sitosterol, campesterol, stigmasterol, cholesterol), the amino acid carnitine and two glucosides of abscicic acid has been reported for *P. americana* seeds (Nwaogu *et al.*, 2008; Takenaga *et al.,* 2008; Wang *et al.,* 2010). High concentrations of catechins, procyanidins and hydroxycinnamic acid have recently been determined in 100% ethyl acetate (EtOAc), in 70% acetone and 70% methanol (MeOH) extracts obtained from *P. americana* peel and seeds, while the pulp extract was rich in hydroxybenzoic acid, hydroxycinnamic acid and procyanidins (Rodríguez-Carpena *et al.*, 2011).

Interestingly, the hypolipemic effect of the MeOH extract obtained from *P. americana* seeds has been demonstrated in male rats with induced hypercholesterolemia (Asaolu *et al.*, 2010; Imafidon and Amaechina, 2010). The same effect was described for the aqueous extract, which also reduced blood pressure both in normal rats and those with high blood pressure; in addition, it exerted a hypoglycemic effect on rats and rabbits with diabetes (Okonta *et al.*, 2007; Ogochukwu *et al.*, 2009; Edem *et al.*, 2009; Kofi *et al.*, 2009). The aqueous extract showed a median Lethal dose (LD_{50}) = 10 g/kg in rats when it was administered orally. Importantly, it did not alter the hematological parameters nor the levels of Alanine amino transferase (ALT), Aspartate amino transferase (AST), albumin, and creatinine in male and female rats were treated for 28 days (Ozolua *et al.*, 2009).

The hexane and MeOH seed extracts of *P. americana* have been described to have a Minimum inhibitory concentration (MIC) of <1.25 µg/ml against *Candida sp.*, *Cryptococcus neoformans* and *Malassezia pachydermatis*. These extracts were also active against *Artemia salina*, with Lethal concentration (LC_{50}) values of 2.37 and 24.13 mg/mL, respectively. They were also active against *Aedes aegypti* larvae with LC50 values of 16.7 and 8.9 mg/mL, respectively (Giffoni *et al.*, 2009). On the other hand, the MeOH extract from *P. americana* leaves inhibited completely the growth of *M. tuberculosis* (MIC = 125 µg/mL) and H37Rv (MIC = 62.5 µg/mL); furthermore, the hexane fraction inhibited the growth of both mycobacteria with MIC = 31.2 µg/mL (Gomez-Flores *et al.*, 2008). In addition, the EtOH extract was active against both Gram positive and negative bacteria (with the exception of *Staphylococcus epidermis* and *Escherichia coli*) with MIC of 500 µg/mL (Raymond-Chia and Dykes, 2011). Regarding the bacterial activity of *P. americana* (var Hass and Fuerte), the acetone seed extract exhibited moderate activity against *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* (Rodríguez-Carpena *et al.*, 2011).

The trypanomicidal activity of the MeOH extract from *P. americana* seeds has been also tested (Abe *et al.*, 2005). It showed moderate activity when it was evaluated at the concentration range of 250–500 µg/mL. In the case of the aqueous seed extract, it had a slight anti-Giardia duodenalis (syn G. lamblia) activity, inducing 23% of mortality at 4 mg/mL (Ponce-Macotela *et al.*, 1994). The antioxidant activity (AOA) of *P. americana* seed, peel, pulp and leaves extract has been described by different methods

(Wang *et al.,* 2010; Rodríguez-Carpena *et al.,* 2011; Matsusaka *et al.,* 2003; Yean-Yean and Barlow, 2004; Asaolu *et al.,* 2010a,b).

Jiménez-Arellanes *et al.* (2013) reported the antiprotozoal and antimycobacterial activities of ethanol and chloroform extracts of *P. americana*.

Analgesic and anti-inflammatory properties of the leaves have been reported by (Adeyemi *et al.,* 2002).

Volatile constituents of avocado mesocarp were isolated by concurrent steam distillation/solvent extraction in the Likens-Nickerson apparatus using pentane ether as solvent. The extracts which resulted were concentrated in a Kuderna-Danish concentrator and analysed using gas chromatography and linked gas chromatography-mass spectroscopy (GC-MS) employing capillary columns of contrasting polarity. Hydrocarbons (mainly sesquiterpenes) and alkanals were the predominant constituents present. In the immediate extract of the avocado mesocarp, β-caryophyllene (60%) was the main sesquiterpene, followed by α-humulene (5.9%), caryophyllene oxide (4.8%), α-copaene (4.5%) and α-cubebene as the main hydrocarbons; alkanals were present, but only in low concentrations (Sinyinda and Gramshaw, 1998).

In the extract prepared following storage (2 h) of the mesocarp at room temperature, β-caryophylene (28.8%) was the main sesquiterpene, followed by α-copaene (10.7%), a cadinene isomer (8.5%), α- and β-cubebene (7.7%) α-famesene (5.3%) and octane (4.8%) as principal hydrocarbons; decenal (6.3%) and heptenal (3.2%) were the main aldehydes (Sinyinda and Gramshaw, 1998).

Investigation of the essential oils composition of some *Persea* spices revealed β-caryophyllene (43.9%) and valencene (16.0%) as the most abundant compounds in the oil of *P. americana* leaf from Nigeria (Ogunbinu *et al.,* 2007). Methyl chavicol (syn., estragole) (53.9%) is the major component in the oil of *P. americana* var. *drymifolia* cv. Duke from Cuba (Pino *et al.,* 2004). (*E*)-Avocadodienofuran (15.3%), (*E*)-avocadenynofuran (13.2%), and β-caryophyllene (11.0%) were the major components in the oil of *P. indica* Spreng (Pino and Rolando, 2006).

In another study, the oil of *P. americana* leaf revealed that (*Z*)-nerolidol, (*E,E*)-2,4-decadienal, (*E,E*)-α-farnesene, β-caryophyllene, caryophyllene oxide, and α-copaene were the major components (Pino *et al.,* 2004).

Larijani *et al.*, (2010) characterized thirty-six components in the oil of *P. americana* leaf from Iran, accounting for 97.7% of the oil. The oil consists of 54.5% monoterpenes, 0.6% oxygenated monoterpenoids, 37.7% sesquiterpenes, and 4.9% oxygenated sesquiterpenoids with methyl eugenol (31.2%), β-caryophyllene (16.9%), estragole (9.0%), δ-cadinene (4.8%), β-pinene (4.2%), and α-pinene (3.2%) as the major components in the oil.

Figure 2.2: Pictures of *P. americana* Leaf, Fruit and Seed

2.8.2 *Carica papaya* (Pawpaw)

Brief Description

Carica papaya Linn belonging to family *Caricaceae* is commonly known as papaya or pawpaw in English (Yogiraj *et al.,* 2014). The papaya is a large, tree-like plant, with a single stem growing from 1.8 to 10 m (6 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruits are borne.

The leaves are large, 50–70 cm, palmately lobed or deeply incised with entire margins and petioles of 1-3 feet in length. Stems are hollow, light green to tan brown in color with diameter of 8 inches (Arvind *et al.*, 2013). The flowers appear on the axils of the leaves, maturing into large fruit, 15–45 cm long and 10–30 cm in diameter. Fruits are borne axillary on the main stem, usually singly but sometimes in small clusters. The fruit, which vary in shape from spherical to elongate and which may weigh from 0.5 up to 20 lbs (9 kg), ripens when it feels soft and its skin has attained amber to orange hue (Figure **2.3**). Flesh is yellow-orange to salmon (pinkish-orange) at maturity (Yogiraj *et al.,* 2014). The edible portion surrounds the large central seed cavity. Individual fruits mature in 5-9 months, depending on cultivator and temperature. Plants begin bearing fruits in 6-12 months (Arvind *et al.,* 2013). Unusually for large plants, the trees are dioecious. The tree is usually unbranched, unless lopped.

Ethnomedicinal /Pharmacological Importance

C. papaya is eaten fresh as breakfast fruit or in salads or desserts. Papaya is also exploited for its latex, which contains papain, a proteolytic (protein-digesting) enzyme used in meat tenderizers.

Traditionally, the leaves have been used for treatment of a wide range of ailments. The leaves are made into tea as a treatment for malaria in some parts of the world (Titanji *et al.,* 2008). Antimalarial and antiplasmodial activity has been noted in some preparations of the plant, but the mechanism is not understood and not scientifical proven. The young leaves of *C. papaya* are steamed and eaten like spinach in some parts of Asia and in the treatment of jaundice, urinary complaints and gonorrhoea, fever and dressing wounds (Krishna *et al.*, 2008). Papaya leaf is dried and cured like a cigar to be smoked by asthmatic persons. An infusion of fresh papaya leaves is used by person to expel or destroy intestinal worms. Additional benefits of papaya leaves include; as an acne medicine, appetite increase, menstrual pain ease, meat tenderizer and nausea reliever (Arvind *et al.,* 2013). The leaves of *Carica papaya* are used in herbal medical practices in South East Nigeria to treat malaria and typhoid fever (Igwe, 2015).

Ripe papaya fruit is used as laxative which assures of regular bowel movement. The folic acid found in papayas is needed for the conversion of homocysteine into amino acids such as cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls, is considered a significant risk factor for a heart attack or stroke (Arvind *et al.*, 2013).

Papaya seed is used as carminative, emmenagogue, vermifuge, abortifacient, counter irritant, paste in the treatment of ringworm and psoriasis, antifertility agents in males. The black seeds are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper (Yogiraj *et al.*, 2014).

Papaya peel is often used in cosmetics as skin lightening agent. When peel is mixed with honey and applied it can act as soothe and moisturizers the skin. The papaya vinegar with lemon juice can be applied to the scalp for 20 minutes prior to shampooing to fight dandruff. Adding papaya oil and vinegar to bathwater, along with essential oils like lavender, orange and rosemary can be nourishing, refreshing and relaxing, and can work as a pain reliever and muscle relaxant (Arvind *et al.*, 2013).

Juice from papaya roots is used in some countries of Asia to ease urinary troubles. A decoction formed by boiling the outer part of the roots of the papaya tree in the cure of dyspepsia (Arvind *et al.*, 2013). The roots are also said to cure piles and yaws.

C. papaya is also applied topically for the treatment of cuts, rashes, stings and burns. *C. papaya* ointment is commonly made from fermented papaya flesh, and is applied as a gel-like paste (Morton, 1987). Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as an herbal medicine for contraception and abortion. Enslaved women in the West Indies were noted for consuming papaya to prevent pregnancies and thus preventing their children from being born into slavery (Morton, 1987). The latex is used locally as antiseptic. Infusion of the root is said to remove urine concretions (Reed, 1979). Latex from the plant is used as dyspepsia cure and can also be applied externally to burns and scalds (Reed, 1979). The flowers have been used for jaundice. In Asia, the latex is smeared on the mouth of the uterus as ecbolic. The root infusion is used for syphilis in Africa. Japanese believe that eating papaya prevents rheumatism (Duke, 1984b). Dietary papaya does reduce urine acidity in humans. The inner bark is used for sore teeth. The latex is used in psoriasis, ringworm and it's prescribed for the removal of cancerous growths in Cuba (Duke, 1984b). It has been reported that the extract of unripe pawpaw possesses anti-sickling and reversal of sickling properties (Oduola *et al.*, 2006). The tea prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005; Ayoola and Adeyeye, 2010).

Carica papaya contains an enzyme known as papain which is present in the bark, leaves and fruit. The milky juice contains many biologically active compounds including chymopapain and papain which is the ingredient that aids digestive system, and again used in treatment of arthritis (Arvind *et al.*, 2013).

Previous Work

Phytochemical analysis of methanol and aqueous extracts of *Carica papaya* aerial parts proves the presence of phytocomponents as flavonoids, tannins, alkaloids, carbohydrates and triterpenes (Khaled *et al.*, 2013). Phytochemical analysis of *Carica papaya* leaf extract revealed the presence of flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) glycosides, saponins, tannins and steroids (Anjum *et al.*, 2013). The leaf and fruit were reported to possess carotenoids namely β- carotene, lycopene,

anthraquinones glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities have also been established.

Different properties of papaya such as antioxidant and free radical scavenging activity, anticancer activity, anti-inflammatory activity, treatment for dengue fever, anti-diabetic activity, wound healing activity and antifertility effects has been studied. Thus *Carica papaya a*cts as a multi faceted plant. It is also imperative to identify the mechanism of the plant compounds and studying the active principle of the extract. Thus, papaya should be included in diet as fruit salads, fruit juice, leaf extract, decoction prepared through papaya leaves, etc. However, including papaya seeds in any of the form should be avoided for young men and pregnant women, since it possess antifertility effects that was demonstrated well in animal models (Natarjan *et al.*, 2014).

Studies of Dr. Sanath Hettige (Pigli and Runja, 2014), who conducted the research on 70 dengue fever patients, said papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver. *Carica papaya* leaf extract was found to increase the platelet count and also to decrease the clotting time in rats. The study aims at determining the possible effects of papaya leaves in thrombocytopenia occurring in dengue fever (Krishna and Thomas, 2014; Soobitha *et al.*, 2013; Fenny *et al.*, 2012; Patil *et al.*, 2013). Recent research on papaya leaf tea extract also demonstrated cancer cell growth inhibition. It appears to boost the production of key signaling molecules called Th1-type cytokines, which help regulate the immune system.

Zunjar V investigated the microscopic evaluation of leaves of *Carica papaya* L. to establish the salient diagnostic features for the leaf. The leaf shows abundant sphaeraphides and rhomboidal calcium oxalate crystals. The leaves show no trichomes and a continuous network of veins. Histochemical tests performed indicate the presence of alkaloids and starch. Physiochemical parameters such as extractive values, ash values and moisture content have also been studied for the leaf (Zunjar, 2011).

The antimicrobial activities of different solvent extracts of *Carica papaya* were tested against the Gram-positive and Gram-negative bacterial strains and fungus by observing the zone of inhibition (Baskaran *et al.*, 2012). Papaya seeds have antibacterial properties and are effective against *E. coli*, *Salmonella* and *Staphylococcus* infections. Only the leaf extracts showed inhibitory effect against *Candida albicans*, whereas stem and root extracts were ineffective. Among the leaf, stem and root extracts, the leaf extract is found to exhibit more antimicrobial activity than the stem and root (Sumathi, 2014).

Khaled *et al.* (2013) research work deals with the evaluation of anti-HIV-1 effect of *Carica papaya* aerial parts polar extracts and also the investigation of the chemical content from the polar extracts of the plant. The methanol and aqueous extracts of *Carica papaya* were tested for their anti-HIV-1 activity using the syncytia formation assay. Methanol and aqueous extracts of *Carica papaya* aerial parts showed activity as anti- HIV-1 agents, both of the extracts therapeutic index (TI) of 5.51 and 7.13 compared with the standard drug. The results have shown that *Carica papaya* methanol and aqueous extracts have drug ability as anti- HIV-1 agents.

Achini *et al.* (2012) investigated that management of thrombocytopenia is by drugs and blood products, both of which are costly. Conversely, Sri Lankan traditional medicine use mature leaf concentrate of *Carica papaya* to treat this condition. This claim was scientifically validated.

Igwe (2015) reported the chemical constituents of the extract of the leaves of *C. papaya* with isopropanol as a choice of solvent. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed six compounds which were identified to be hexahydro-1-aH-naphtho[1,8a-b]oxiren-2(3H)-one (2.17%), 3,7-dimethyloct-7-en-1-ol (8.08%), 3-methyl-4- (phenylthio)- 2-enyl- 2,5-dihydrothiophene-1, 1-dioxide (11.78%), cyclopentane undecanoic acid methyl ester (12.02%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (37.78%) and 9-octadecenamide (28.18%). The extract showed potent antimicrobial activity against *Staphylococcus aureus*, *streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. Highest sensitivity was shown with *S. aureus* (14.33 mm at 100% concentration) followed by *E. coli* (12.98 mm at 100% concentration) and *P. mirabilis* (12.37 mm at

100% concentration) while the least was shown with *S. faecalis* (11.39 mm at 100% concentration).

Figure 2.3: Pictures of *C. papaya* Tree, Fruit and Seed

2.8.3 *Ananas comosus* (Pineapple)

Brief Description

Pineapple with scientific name *Ananas comosus* (Figure **2.4**) is a tropical plant with edible multiple fruit consisting of coalesced berries, named for resemblance to the pine cone, is the most economically important plant in the *Bromeliaceae* family (Coppens d'Eeckenbrugge and Freddy, 2003).

The pineapple is an herbaceous perennial which grows to 1.0 to 1.5 meters (3.3 to 4.9 ft) tall, although sometimes it can be taller (Coppens d'Eeckenbrugge and Freddy, 2003). In appearance, the plant itself has a short, stocky stem with tough, waxy leaves. When creating its fruit, it usually produces up to 200 flowers, although some large-fruited cultivars can exceed this. Once it flowers, the individual fruits join together to create what is commonly referred to as a pineapple. After the first fruit is produced, side shoots (called 'suckers' by commercial growers) are produced in the leaf axils of the main stem. Commercially, suckers that appear around the base are cultivated. It has 30 or more long, narrow, fleshy, trough-shaped leaves with sharp spines along the margins that are 30 to 100 centimeters (1.0 to 3.3 ft) long, surrounding a thick stem. In the first year of growth, the axis lengthens and thickens, bearing numerous leaves in close spirals. After 12 to 20 months, the stem grows into a spike-like inflorescence up to 15 cm long with over 100 spirally arranged, trimerous flowers, each subtended by a bract (Davidson, 2008). Flower colors vary, depending on variety, from lavender, through light purple to red.

The ovaries develop into berries which coalesce into a large, compact, multiple accessory fruit. The fruit of a pineapple is arranged in two interlocking helices, eight in

one direction, thirteen in the other, each being a Fibonacci number (Jones and William, 2006).

Ethnomedicinal / Traditional Importance

Pineapple has been used as a medicinal plant in several native cultures. It may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs, dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. In addition to consumption, in the Philippines the pineapple's leaves are used as the source of a textile fiber called piña, and is employed as a component of wall paper and furnishings, amongst other uses (Bartholomew *et al.*, 2003; Davidson, 2008).

The whole plant is used to treat typhoid fever in Ijebu Ode Local Government Area in Ogun State of Nigeria (Fadimu *et al.,* 2014). Roasted unripe fruit juice is used by different communities of Gohpur of Sonitpur district, Assam, India for strangury; a condition caused by blockage or irritation at the base of the bladder, resulting in severe pain and a strong desire to urinate (Saikia, 2006). The *Garo* tribal community of Netrakona district in Bangladesh uses fruit juice for fever and leaf juice for helminthiasis and jaundice (Rahmatullah *et al.,* 2009). The root and fruit are either eaten or applied topically as an anti-inflammatory, digestive and proteolytic agent (Bhakta *et al.* 2012). It is traditionally used as an antiparasitic and anthelmintic agent in the Tripura. This fruit can also be used to aid digestion. A root decoction is used to treat diarrhoea**.** In some cultures, pineapple has become associated with the notion of welcome, an association bespoken by the use of pineapple motifs as carved decorations in woodworking. It can clear bronchial passages in those suffering with pneumonia and bronchitis. The anti-inflammatory properties in this fruit help reduce the symptoms of arthritis, and help reduce pain after surgery and sport injuries (Hossain *et al.,* 2015). Pineapple is currently being studied for its effectiveness in preventing heart disease.

Previous Work

Research reported in the December 2005 edition of "Medical Science Monitor" studied the effect of pineapple and other fruit juices on plasma lipids. Researchers discovered that rats that consumed pineapple juice over a three-hour period experienced a decrease in lipoprotein particles, compounds that carry fat through the blood, and increased metabolism, activities that lower cholesterol levels. The April 2011 "Journal of Medicinal Food" revealed that the bromelain in pineapple juice slightly increased

the quantity of tendon cells after a crush injury to the Achilles heel in rat models; it also decreased the levels of malondialdehyde, a compound that may cause mutations in tissues. In doing so, pineapple juice contributed to healing in the early stages of an injury (Bhakta *et al.,* 2012).

The July-August 2009 issue of "Oxidative Medicine and Cellular Longevity" features a study from Indian researchers, which correlates manganese consumption in pineapple juice and other foods with increased sperm movement. It also protected sperm during freezing for storage, which can raise your chances of conception (Bhakta *et al.,* 2012; Debnath *et al.,* 2012).

More than 280 volatile compounds had been identified among the aroma volatiles of pineapple, whereas only a few volatiles contribute to pineapple aroma (Tokitomo *et al.,* 2005). It has been reported that esters were the most abundant pineapple volatiles, in particular, ethyl hexanoate and methyl hexanoate which have the highest contribution to the pineapple aroma (Pickenhagen *et al.,* 1981; Morais and Silva, 2011). Marta *et al.* (2010), Elss *et al.* (2005), Taivini *et al.* (2001), Umano *et al.* (1992), Akioka *et al.* (2008) also reported that esters were the major volatile compounds in pineapple volatile composition, however, He *et al.* (2007) reported that hydrocarbons and esters were the main compounds, which could be explained by differences in cultivars, growing conditions, and volatiles extraction methods. Such differences could also justify why methyl butanoate and methyl 2-methylbutanoate were not found in 'Smooth Cayenne' pineapple, despite being the main components in other studies (Wei *et al.*, 2011).

On the other hand, Berger (1991) reported two minor hydrocarbon compounds, 1-(*E,Z*)-3,5-undecatriene and 1-(*E,Z,Z*)-3,5,8-undecatetraene as the important contributors to fresh-cut pineapple aroma due to their low odor threshold values. Also the results of Takeoka *et al.* (1991) reported many sulfur-containing esters among pineapple volatiles.

Wei *et al.* (2011) identified 44 volatile compounds when characterizing pineapple pulp and core by headspace-solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) and revealed the presence of methyl hexanoate, ethyl hexanoate, methyl 3-(methylthio) propanoate, methyl octanoate, ethyl decanoate, α-terpineol, nonanal, and decanal.

According to Facundo (2009), the compound ethyl hexanoate is related to the aroma note described as 'pineapple'. This compound was also identified in the pineapple processing residue distillate obtained by simple hydrodistillation technique.

Tokitomo *et al.* (2005) prepared an aroma distillate from fresh pineapple using solvent-assisted flavor evaporation and detected 29 odor-active compounds. Elss et al. (2005) reported the presence of the following volatile compounds when characterizing aroma of fresh pineapple juice and its water phase extracts: 2-methyl-3-buten- 2-ol, methyl pentanoate, butyl acetate, hexanal, 2-pentanol, 1-butanol, ethyl hexanoate, limonene, z-ocimene, linalool, furfural, acetic acid, α-terpineol, geraniol, and γ-octalactone.

Figure 2.4: Pictures of *A. comosus* Fruit

2.8.4 *Theobroma cacao* Linn (Cocoa)

Brief Description

Theobroma cacao L. belongs to the genus of flowering plants in the mallow family, *Malvaceae*, which is sometimes classified as a member of *Sterculiaceae*. It contains roughly 20 species cultivated in the American, African, and Asian continents, and many countries worldwide are involved in cocoa production, marketing, and consumption (Almeida and Valle, 2007, 2009; Bertolde *et al.*, 2014). Several species of *Theobroma* produce edible seeds, notably cacao, cupuaçu, and mocambo. The seeds usually inside the fruit; called a cocoa pod (ovoid) which is 15–30 cm long and 8–10 cm wide, ripening yellow to orange as seen in Figure **2.6**, and weighs about 500 g when ripe. The pod contains 20 to 60 seeds, usually called "beans", embedded in a white pulp (Izuka and Mbagwu, 2013).

Ethnomedicinal importance/ Previous work

Cocoa has been extensively worked on with literature report on the various use to alleviate fever, shortness of breath and heart conditions and manuscripts produced in Europe and New Spain from the 16th to early 20th century revealed more than 150 medicinal uses for cocoa and/or chocolate (Dillinger *et al.*, 2000, Cooper *et al.*, 2008).

Cocoa bean has been reported to have anti-aging properties. The leaf and seeds *Theobroma cacao* lowers blood sugar, fatigue, kidney malfunction and serve as anti-ulcer and tumor. Cocoa bean was also reported by Sharma *et al.* (2012) to be good for anxiolytic action. Cacao pigment, which is extracted from the husks, has shown anti-HIV properties. The substance, consisting of polymerised flavonoids (e.g. catechins, anthocyanidins and leukoanthycyanidin) inhibits the cytopathic effects of HIV in cell

culture. It appears to inhibit absorption of the virus rather than limiting its replication once it is absorbed (Unten *et al.,* 1991).

The stem bark of *Theobroma cacao* is boiled with water and mixed with hot pap as baby food in the treatment of anaemia (Gbadamosi *et al.,* 2012). Cocoa pod is a waste product of cocoa seeds and have been found very useful industrially for making black soaps which are highly medicinal for treating various ailment (Adewole *et al.,* 2013). This means that regular consumption of cocoa will reduce the occurrence of malaria attack (Jayeola *et al.,* 2011). Cocoa pod was also reported to be a good precursor for active carbon (Adeyi, 2010).

The antioxidant capacity of cocoa bean and leaves has been investigated by Othman *et al.* (2007) and Osman *et al.* (2004)

The plant was found to be rich in active metabolites like flavonoids, alkaloids, phenols, saponin and tannins in the leaf, stem bark and seed as shown in Table **2.4**

Fapohunda and Afolayan (2012) also reported the presence of phenols and tannins in cocoa pod husk.

The analytical methodologies applied to isolate and purify cocoa bioactives involve laborious pretreatment together with isolation and purification procedures (Hatano *et al.,* 2002; Ortega *et al.,* 2008; Stark and Hofmann, 2005). Among these, isolation by semi-preparative and preparative liquid chromatography (LC) with C18 reversed phase (RP) offers high versatility to separate a wide range of nitrogenous and non-nitrogenous bioactive compounds (Contreras *et al.,* 2009; Rzeppa *et al.,* 2011; Stark and Hofmann, 2005).

The usual technique to analyze polyphenols from cocoa multicomponent extracts or a specific isolated fraction is reversed phase high-pressure liquid chromatography (RP-HPLC) with C8 (Srdjenovic *et al.,* 2008), C12 (Pereira-Caro *et al.,* 2013) and C18 (Andres-Lacueva *et al.,* 2008; Calderón *et al.,* 2009; Quiñones *et al.,* 2011; Tomas-Barberán *et al.,* 2007) stationary phase. As solvent system, all of these authors used linear gradients with acidified water (using formic or acetic acid) and acetonitrile or methanol as organic solvent. This separation technique has been coupled to different detectors for the qualitative and quantitative characterization of these compounds, such

as ultraviolet and diode-array detection (DAD) (Quiñones *et al.,* 2011; Srdjenovic *et al.,* 2008), fluorescence (Payne *et al.*, 2010; Pereira-Caro *et al.*, 2013) and/or mass spectrometry (MS) (Andres-Lacueva *et al.*, 2008; Ortega *et al.*, 2010; Pereira-Caro *et al.,* 2013).

Amongst the phytochemicals which are majorly polyphenols isolated from cocoa bean include;

Flavonoids – Quercetin glucuronide, Quercetin hexose, Hexenyl xylopyranosyl glucopyranoside, Quercetin arabinoside, Quercetin, Sucrose, Tri-O-methylsucrose, Epicatechin **(2.36)**, catechin **(2.37)**, Procyanidin **(2.38)**, (Epi)catechin glucopyranoside, (Epi)gallocatechin, Catechin diglucopyranoside, Proanthocyanidin Arabinopyranosyl-(epi)catechin, (Epi)catechin pentamer, (Epi)catechin dimer hexose, (Epi)catechin methyl dimer, trans-Clovamide (*N*-[(2E)-3-(3.4-dihydroxyphenyl)-1-oxo-2-propen-1-yl]-3-hydroxy-L-tyrosine), Deoxyclovamide (*N*-[(2E)-3-(3.4-Dihydroxyphenyl)-1-oxo-2-propen-1-yl]-L-tyrosine) (Cádiz-Gurrea *et al.,* 2014).

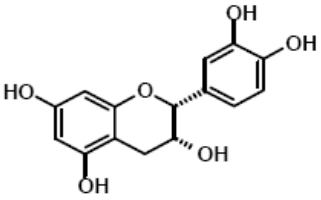
Alkaloids – Theobromine **(2.39)** (Cádiz-Gurrea *et al.*, 2014), Caffeine **(2.40)** (Zheng *et al.,* 2002), Theophyline **(2.41)** (Abbe Maleyki and Amin, 2008).

Analysis of essential oils of the leaves of two cocoa clones by GC and GC-MS revealed twenty-four and twenty compounds which were identified to be aldehydes, ketones, alcohols and fatty acids (Chee *et al.*, 2005).

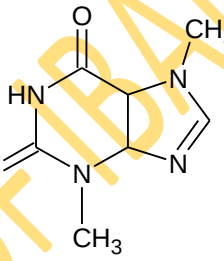
To date, essential oils of other parts of the plant have not been investigated to the best of my knowledge.

Table 2.4: Reported Phytochemicals of *T. cacao* Plant parts

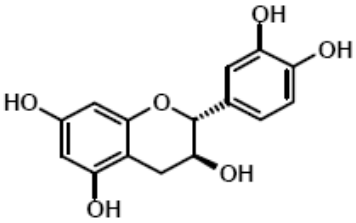
Sample	Sap	Tannin	Phenol	Flav	Alk	Triter	Ref
Leaf	+	+	+	+	+	+	Zainal *et al.*, 2014
Stem bark	+	-			+	+	Ogunmefun *et al.*, 2013
	+	+	+	+	+		Nwokonkwo and Okeke, 2014
Seed	+	+	+	+	+	+	Izuka and Mbagwu, 2013



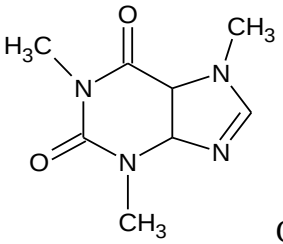
(-)-Epicatechin **2.36**



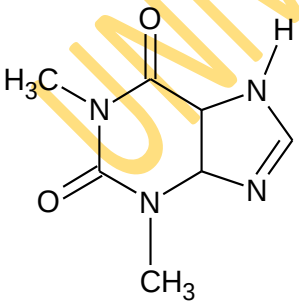
Theobromine **2.39**



(+)-Catechin **2.37**

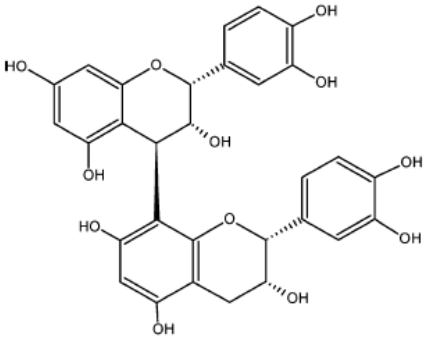


Caffeine **2.40**

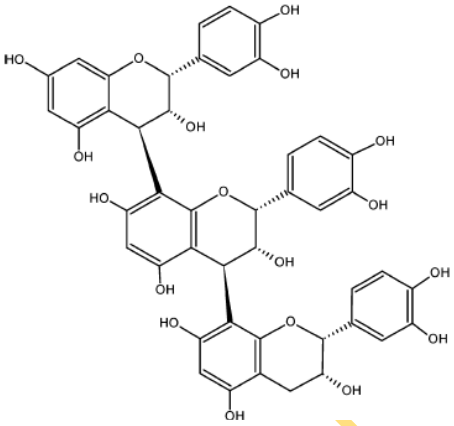


Theophyline **2.41**

Procyanidin Dimer and Trimer in Cocoa 2.38



Dimer B2, epicatechin-(4β-8)-epicatechin



Trimer C1, epicatechin-(4β→8)]2-epicatechin

Figure 2.5: Structures of Some Isolated Compounds in Cocoa

Source: Cadiz-Gureira *et al.*, 2014

Figure 2.6: Pictures of *T. cacao* L. Tree and Pod Containing Seed

2.8.5 *Chrysophyllum albidium* G. Don (African Star Apple)

Brief Description

African star apple (*Chrysophyllum albidum* G. Don) is a tropical edible fruit tree. It belongs to the family of *Sapotaceae* which has up to 800 species and make up almost half of the order (Ehiagbonare *et al.*, 2008). It is primarily a forest tree species and its natural occurrences have been reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). The plant often grows to a height of 36 m though it may be smaller (Keay, 1989). The African star apple fruit is a large berry containing 4 to 5 flattered seeds or sometimes fewer due to seed abortion as seen in Figure **2.7**. The leaves are oval, green above, densely golden pubescent below from which the genus is named (Figure **2.7)**. The plant has in recent times become a crop of commercial value in Nigeria (Oboh *et al.*, 2009).

The fruit is commonly found in the Central, Eastern and Western Africa. It is a popular tropical fruit tree and widely distributed in the low land rain forest zones and frequently found in villages (Okoli and Okere, 2010). It has common names known as *agbalumo* (Yoruba), *udala* (Igbo), *agbaluba* (Hausa) and *eha* (Ebira) in the local

languages in Nigeria (Amusa *et al.*, 2003). *C. albidum* fruit is common in both urban and rural centres especially during the months of December to April. The fruits are not usually harvested from the trees, but left to drop naturally to the forest floor where they are picked up (Amusa *et al.*, 2003). It has sweet edible fruits and various ethnomedical uses (Adebayo *et al.*, 2011).

Ethnomedicinal /Traditional Use

Amusa *et al.* (2003) pointed out that across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethnomedicinal uses. *C. albidum* is widely used as an application to sprains, bruises and wounds in herbal medicine in southern Nigeria. The seeds and roots extracts of *C. albidium* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerates wound healing process (Okoli and Okere, 2010). The people of south western Nigeria have been using *C. albidum* leaves for the management of infections and ailments since prehistoric times (Duyilemi and Lawal, 2009). The roots and leaves of *C. albidum* have been widely used for medicinal purposes (Adewusi, 1997). In addition, its seeds are a source of oil, which is used for diverse purposes (Ugbogu and Akukwe, 2009). *C. albidum* is used in folklore in the treatment of yellow fever, malaria, diarrhea, vaginal and dermatological infections (Adebayo *et al.*, 2011). The bark is used for the treatment of malaria and yellow fever (Adebayo *et al.*, 2011), while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea (Idowu *et al.*, 2006) which are as a result of infections and inflammatory reactions (Adisa, 2000; Idowu *et al.*, 2006). The leaf extract of *C. albidum* can help to thin the blood (antiplatelet effect) as well as regulate the sugar level in blood sugar (Adebayo *et al.*, 2010). The root bark has been known to have antifertility effect on the male (Onyeka *et al.*, 2012). *Chrysophyllum albidum* is established to have haematinic potentials (Adewoye *et al.*, 2012). The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used as a remedy for yellow fever and malaria (Duyilemi and Lawal, 2009). The cotyledons from the seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria. The seeds are also used for local games or discarded (Bada, 1997).

C. albidum is good for the treatment of fibroids as reported by Egunyomi and Oladunjoye (2012). When freshly harvested the fleshy and juicy fruits have potentials as an ingredient of soft drinks and can be fermented for wine or other alcohol production (Ajewole and Adeyeye, 1991).

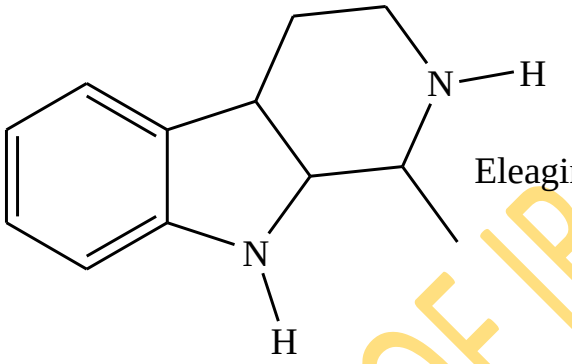
Previous Work

Phytochemical profile shows that African star apple leaves contain an array of biologically active substances that include alkaloids, tannin, saponin, phenol and flavonoid (Amusa *et al.,* 2003; Okoli and Okere, 2010; Orijajogun *et al.* 2013; Kamba and Hassan, 2011). African star apple fruits contain crude protein content of 8.75%, carbohydrate content of 29.6% and moisture content of 42.1% as reported by Amusa *et al.,* 2003. The fleshy pulp of the fruits is eaten especially as snack and its fruit has been found to have higher contents of ascorbic acid than oranges and guava (Amusa *et al.,* 2003). It was also reported as an excellent source of vitamins, irons, flavours to diets (Adisa, 2000).

Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related diseases such as; diabetics, cancer and coronary heart diseases (Burits and Bucar, 2002). In fact, the effect of DPPH free radical scavenging activity on the fractions of petroleum ether, ethanol, butanol, ethyl acetate and water extracts of the leaves was determined. The ethyl acetate fraction was purified in column chromatography to obtain myricetin rhamnoside which also exhibited an excellent radical scavenging activity compared with the standard or positive control as studied by Adebayo *et al.* (2011).

Previous research on *C. albidum* include seed storage and its food value, physical properties of the seed, use of the shell of seeds for the removal of metal ions and antimicrobial effect of oil from its seeds against some local clinical bacteria isolates (Amusa *et al.,* 2003; Oyelade *et al.,* 2005; Ugbogu and Akukwe, 2008; Oboh *et al.,* 2009). Eleagnine **(2.42)**: an alkaloid isolated from the seed cotyledons has also been examined for its antinociceptive, anti-inflammatory and antioxidant activities (Idowu *et al.,* 2006) and antiplatelet effect by Adebayo *et al.* (2010). The stem bark has antimicrobial activity (Adewoye *et al.,* 2011). The hydrogalacturonic acid (pectin) content of the star apples is low (Alawuba *et al.,* 1994).

In 2001, Moronkola identified eight (8) compounds accounting for 90.8% of total components with esters (65.1%) constituting the most abundant class of compounds in the essential oil composition of *C. albidum* fruit. A phthalate (dibutyl-1,2-benzenedicarboxylate) was reported as the major compound. GC and GC-MS analysis of the root essential oil had twenty-four (24) compounds. Monoterpenes (40.5%) and sesquiterpenes (27.9%) were the dominant class of compounds with pinene (34%), caryophyllene (12.8%), isocaryophyllene (8.5%) and 1,8-cineole (6.5%) as the major compounds (Moronkola *et al.*, 2006).



Eleaginine **2.42**

Figure 2.7: Pictures of *C. albidum* Leaves, Seed and Fruit

CHAPTER 3

MATERIALS AND METHODS

3.1 General Experimental Procedures

All solvents were of analytical grade and used as supplied. Column chromatography was performed on silica gel (Merck 70-230 mesh). Thin layer chromatography (TLC) was performed on aluminium plates coated with silica gel (Merck 60 mesh). TLC bands were visualized under ultraviolet light (at 254 nm and 365 nm) and by spraying with cerric sulphate using gun spray. Solvents were removed under reduced pressure using a Buchi rotary evaporator at pump pressure of 0.1 mmHg.

^{1}H and ^{13}C NMR were recorded at 600 MHz on Bruker Avance spectrophotometer at 300 K in deuterated solvents. Chemical shifts were expressed in parts per million (ppm). Tetramethylsilane (TMS) was used as internal reference for ^{1}H resonances and were referred to the solvent peaks (chloroform-*d*; δ_H 7.25 for residual $CDCl_3$, and δ_C 77.0). EI mass spectra were recorded on Varian MAT 312 double focusing spectrophotometer while IR spectra were recorded in chloroform on a Perkin-Elmer 580 FTIR spectrophotometer. Melting points were determined on Yanaco micromelting point apparatus and were uncorrected.

3.2 Plant Collection and Identification

Fresh plant parts of *Persea americana*, *Carica papaya*, *Chrysophyllum albidum* and *Theobroma cacao* (Table **3.**1) were collected from farms located at the outskirts of Ibadan towards Ikire. *Ananas comosus* fruits were purchased from Oje Market, Ibadan. Authentication of all the plant samples was carried out at Forest Research Institute of Nigeria (FRIN). Voucher specimens were duly deposited in the FRIN herbarium and voucher numbers assigned (Table **3.2**).

Table 3.1: Plant Parts and Code

S/N	Plant Sample	Part used	Code
1	*Persea americana* Mill.	Stem Bark	PASB
		Root Bark	PARB
		Leaf	PAL
		Peel	PAP
		Fruit	PAF
		Seed	PASE
2	*Carica papaya*	Root Bark	CPRB
		Root	CPR
		Stem Bark	CPSB
		Stem	CPS
		Leaf	CPL
		Fruit	CPF
		Seed	CPSE
		Peel	CPP
3	*Ananas comosus*	Shoot	ACSH
		Peel	ACP
		Fruit	ACF
4	*Theobroma cacao* Linn.	Stem Bark	TCSB
		Leaf	TCL
		Pod	TCP
		Seed	TCSE
5	*Chrysophyllum albidum*	Stem Bark	CASB
		Root Bark	CARB
		Leaf	CAL
		Seed	CASE
		Seed Bark	CASeB
		Fruit Bark	CAFB

Table 3.2: Voucher Number of Selected Samples

S/N	Plant Name	Voucher Number
1	*Persea americana* Mill	FHI 110501
2	*Carica papaya* Linn	FHI110500
3	*Ananas comosus* (L) Merr	FHI110495
4	*Theobroma cacao* Linn	FHI110502
5	*Chrysophyllum albidum* G. Don	FHI110499

3.3 Extraction of Plant Materials

3.3.1 Hydrodistillation

Fresh matured plant parts of the fruit samples were air-dried and 200–400 g of each dried sample was subjected to hydrodistillation using an all-glass Clevenger-apparatus designed by the British Pharmacoepoeia Specifications (1980) for 4 hours using a 5 L quick fit round bottom flask. The oils were dried in desiccators containing anhydrous sodium sulphate (Na_2SO_4) for 24 h and then stored in airtight vials in a refrigerator at 4 ^oC. The yields were calculated according to the weight of the plant material before distillation.

3.3.2 Extraction of Non-Volatile Components

The air-dried, pulverized fruit plant parts were extracted with methanol by 72 hours maceration method as outlined in Scheme **3.1**. Maceration was done thrice for each sample to ensure exhaustive extraction and maximum yield. The extracts were decanted and double filtered using cotton wool and whatmann No. 1 filter paper. All the filtered extracts were concentrated on rotary evaporator at 37 ^oC and dried in the dessicator. The dried crude of each sample was weighed and the yields were calculated according to the weight of the plant material before maceration.

3.4 Determination of Chemical Components of Volatile Extracts

3.4.1 Chromatographic Analyses of Essential Oil

Gas Chromatography (GC)

The oils were analyzed on an Agilent Model 7890A Gas Chromatography equipped with a HP-5ms fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Analytical conditions were: Oven temperature: 60 ^oC, with 2 minutes initial hold, and then to 280 ^oC at 4 ^oC/min, with final hold time of 10 minutes; helium was used as carrier gas at a flow rate of 1 mL/min. Retention indices were determined with reference to a homologous series of normal alkanes analyzed under the same conditions. Percentage composition of each constituent was calculated by integration of the GC peak areas.

Gas Chromatography-Mass Spectrometry (GCMS)

GC-MS analyses were performed on an Agilent Model 7890A Gas Chromatography interfaced to an Agilent 7000 GC/MS Triple Quad. The temperature program used for

the GC was the same as described above. The MS was operated in EI mode with ionization voltage 70 eV and ion source temperature, 250 ^{o}C.

3.4.2 Components identification

The components of the essential oil were identified on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 2007; Joulain and Koenig, 1998) and those of reference compounds from the Library of National Institute of Standard and Technology (NIST, 2011) database.

3.5 Determination of Chemical Components of Non-Volatile Extracts

3.5.1 Phytochemical Screening of Non-Volatile Extracts

Preliminary qualitative phytochemical screening was performed on the non volatile extracts using standard procedures to identify chemical constituents.

Screening for alkaloids

The extract (0.5 g) was stirred in 5 mL of 1% dilute HCl on water bath and filtered while hot. One mL of the filtrate was treated with a few drops of Dragendorff's reagent. An orange brown precipitate was taken as evidence for the presence of alkaloids in the extract (Evans, 2002).

Screening for flavonoids

Five millilitres of dilute ammonia solution was added to a portion of the plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing (Edeoga *et al.*, 2005)

Screening for tannins

Two millilitre of the extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Savithramma *et al.*, 2011).

Screening for saponins

Five millilitres of extract was mixed with 20 mL of distilled water and then agitated for 15 minutes. Formation of foam indicated the presence of saponins (Kumar *et al.*, 2009).

Screening for glycosides

Five millilitres of the extracts were treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Concentrated sulphuric acid was added, a reddish brown colouration at the junction of the two layers and bluish green colour in the upper layer indicated the presence of glycosides (Siddiqui and Ali, 1997).

Screening for Anthraquinones (Borntrager's test)

Each plant extract (5 g) was shaken with 10 mL benzene, filtered 5 mL of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a red colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl anthraquinones (Evans, 2002).

Screening for Anthocyanins

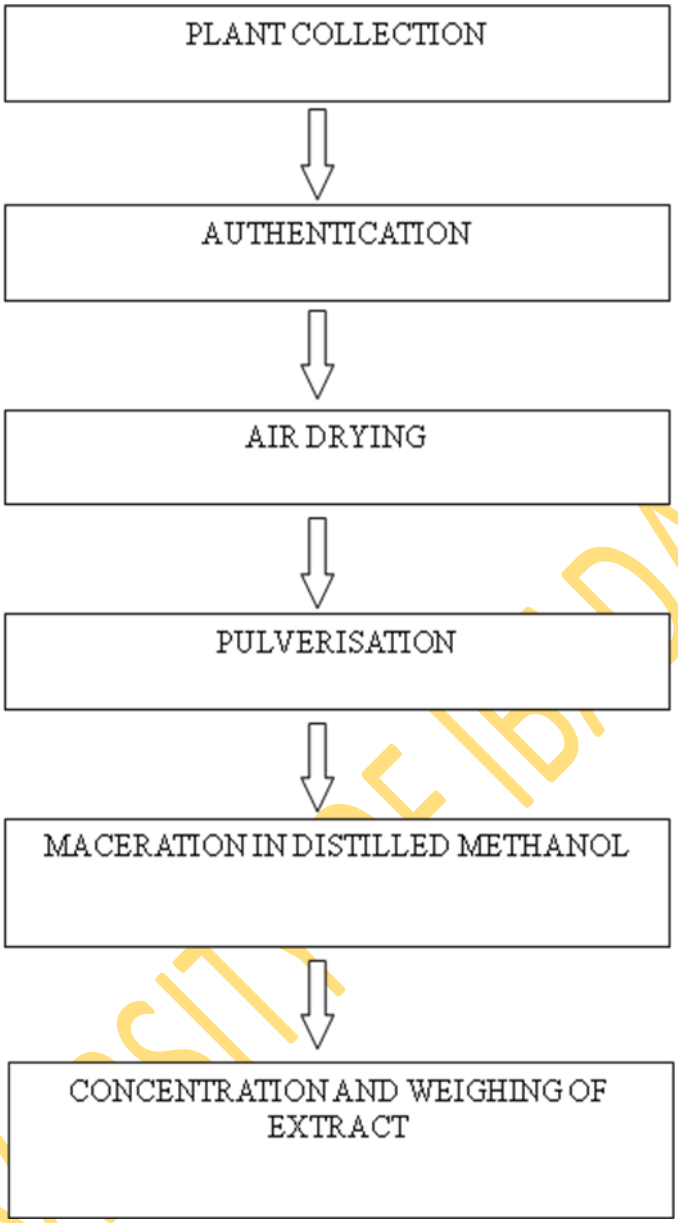
Two millilitres of aqueous extract was added to 2 mL of 2 N hydrochloric acid and ammonia. The appearance of pink-red to blue-violet indicated the presence of anthocyanins (Savithramma *et al.*, 2011).

Screening for Reducing Sugar

The test extract/fraction (0.5 g) was boiled with 10 mL of distilled water and filtered. Two millilitre of 1:1 v/v mixture of Fehling solutions A and B was added to the filtrate and boiled in a water bath for about 3 minutes. Formation of a brick red precipitate indicates the presence of free reducing sugars (Sofowora, 1993).

Salkowski test for steroidal nucleus

The chloroform filtrate (2 mL) of the test extract/fraction was transferred into a clean dry test tube. Using a dropping pipette, 1 mL of conc. H_2SO_4 was then poured carefully down the wall of the test tube to form two layers. A reddish- brown ring at the interface of the two liquids indicates the presence of a steroidal ring.



Scheme 3.1: Isolation Scheme for Non-Volatile Extract

3.6 Isolation of Compounds from *Theobroma cacao* Linn Pod

The methanol extract (25 g) of *T. cacao* L. pod was dissolved in methanol, pre-adsorbed on silica gel (50 g) and mixed to obtain a homogenous solid mixture which was allowed to air-dry in a fume cupboard. The mixture was then loaded on a glass chromatography column packed with silica gel. The column was eluted with the following mobile phase gradient in increasing order of polarity (Scheme **3.2**): n-hexane (100%, 3 L), n-hexane: CH_2Cl_2 [(9:1, 1 L); (4:1, 1 L); and (1:1, 1 L)], CH_2Cl_2 (100%, 1 L), CH_2Cl_2: ethyl acetate (1:1, 1 L), ethyl acetate (100%, 1 L), ethyl acetate: methanol (1:1, 1 L), methanol (100%, 1 L), methanol: water (1:1, 1 L) and water (100%, 1 L). A total of 260 fractions (50 mL portions) were collected and pooled to 30 sub-fractions coded TCH-1, TCHD 1-3, TCD 1-2, TCDE 1-4, TCE-1, TCEM 1-8, TCM 1-4, TCMW 1-6 and TCW-1 based on TLC pattern. Fractions were concentrated to dryness using rotary evaporator and were transferred into weighed and labelled sample bottle. TCHD-3 (75 mg) was further purified on HPLC (LC-980) using 2% n-hexane: ethylacetate solvent system to yield white solid coded 2TCHD-3 (2.6 mg). Sub-fractions TCDE-1 (300 mg) and TCDE-3 (500 mg) were however subjected to column chromatography with 15% and 30% stepwise gradient of n-hexane: acetone solvent system to produce white solids coded 72TCDE-1 (48.6 mg) and 359TCDE-3 (11 mg) respectively.

3.6.1 Purification of 2TCHD-3 Using Chromatography

Hexane: DCM soluble fractions were pooled together to give three sub-fractions (TCHD-1, TCHD-2 and TCHD-3) based on thin layer chromatography (TLC) on precoated aluminium plates. TCHD-3 (75 mg) was purified on HPLC (LC-908) equiped with refractive index indicator using 2% n-hexane: acetone solvent sysytem to yield 2TCHD-3 (white solid). 2TCHD-3 was characterised using spectroscopic techniques.

3.6.2 Characterisation of 2TCHD-3 Using Spectrometry

Appearance: white solid

Melting Point: 112-114 ^{o}C

Molecular formula: $C_{30}H_{48}O_2$

Molecular Mass (EIMS) [M^{+}-H_2O]: 424.3903 g/mol.

Molecular Mass (calculated): 440.3642 g/mol.

Major EIMS m/z fragmentation peaks m/z (relative abundance): 424 (27) [M^+-H_2O], 313 (16), 217 (12), 205 (17), 203 (12), 189 (13), 187 (13), 175 (30), 173 (27), 163 (24), 161 (32), 159 (22), 149 (42), 147 (55), 135 (45), 133 (52), 121 (63), 119 (54), 109 (71), 107 (69), 95 (87), 93 (69), 81 (71), 69 (100), 67 (67), 55 (90).

^{13}C-NMR (600 MHz, $CDCl_3$, δ ppm): 33.5 (t, C_{-1}), 37.5 (t, C_{-2}), 216.7 (s, C_{-3}), 50.2 (s, C_{-4}), 48.4 (d, C_{-5}), 21.5 (t, C_{-6}), 28.1 (t, C_{-7}), 47.9 (d, C_{-8}), 21.1 (s, C_{-9}), 26.0 (s, C_{-10}), 26.7 (t, C_{-11}), 33.4 (t, C_{-12}), 45.3 (s, C_{-13}), 48.7 (s, C_{-14}) 35.5 (t, C_{-15}), 25.85 (t, C_{-16}), 52.1 (d, C_{-17}), 18.1 (q, C_{-18}), 29.6 (t, C_{-19}), 35.7 (d, C_{-20}), 18.3 (q, C_{-21}), 33.2 (t, C_{-22}), 32.7 (t, C_{-23}), 67.6 (d, C_{-24}), 144.3 (s, C_{-25}), 114.3 (t, C_{-26}), 17.0 (q, C_{-27}), 22.2 (q, C_{-28}), 20.8 (q, C_{-29}), 19.3 (q, C_{-30}).

The results are presented in Table **4.9.**

^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm) : 1.52/1.85 (2H, t, $H_{-1a/b}$), 2.27/ 2.3 (2H, t, $H_{-2a/b}$), 1.69 (1H, t, H_{-5}), 0.9/1.5 (2H, q, $H_{-6a/b}$), 1.2 (2H, q, H_{-7}), 1.56 (1H, t, H_{-8}), 2.02 (1H, t, H_{-11}), 1.5 (2H, t, H_{-12}), 1.29/1.24 (2H, t, $H_{-15a/b}$), 1.36 (2H, q, H_{-16}), 1.58 (1H, q, H_{-17}), 0.96 (3H, s, H_{-18}), 0.78/0.55 (2H, $H_{-19a/b}$), 1.29 (1H, m, H_{-20}), 0.87 (3H, d, H_{-21}), 1.64 (2H, q, H_{-22}), 1.63/1.82 (2H, q, H_{-23}), 4.34 (1H, t, H_{-24}), 4.87/4.98 (2H, s, $H_{-26a/b}$), 1.78 (3H, s, H_{-27}), 1.03 (3H, s, H_{-28}), 1.08 (3H, s, H_{-29}), 0.88 (3H, s, H_{-30}).

The results are presented in Table **4.9**.

FTIR spectrum (V_{max} cm^{-1}, KBr): 2941 (C-H), 2869, 1706 ($C=O_{(ketone)}$), 1458, 1375, 1110.9.

3.6.3 Purification of 72TCDE-1 Using Chromatography

DCM: Ethyl Acetate soluble fraction were pooled together to give four sub-fractions (TCDE-1, TCDE-2, TCDE-3 and TCDE-4) based on thin layer chromatography (TLC) on precoated aluminium plates. TCDE-1 (300 mg) was later subjected to column chromatography on silica gel (70-230 mesh) eluted with 15% stepwise gradient of n-hexane: acetone solvent sysytem. 150 fractions were collected in 20 mL portions. Portion 72-79 (white solid) was labelled 72TCDE-1 and subjected to spectroscopic analysis to characterise.

3.6.4 Characterisation of 72TCDE-1 Using Spectrometry

Appearance: white solid

Melting point: 110.4 - 112.9 ^{o}C

Molecular formula (HREIMS): $C_{29}H_{50}O$

Molecular Mass (HREIMS): 414.2 g/mol.

Molecular Mass (calculated): 414.7 g/mol.

Major EIMS m/z fragmentation peaks m/z (relative abundance): 414 (100) [M^+], 412 (43), 396 (43), 381 (24), 329 (31), 303 (33), 273 (28), 231 (21), 213 (32), 163 (22), 159 (32), 144 (36), 134 (22), 118 (23), 108 (22), 95 (35), 69 (32), 55 (41).

^{13}C-NMR (600 MHz, $CDCl_3$, δ ppm): 37.22 (t, C_{-1}), 31.64 (t, C_{-2}), 71.79 (d, C_{-3}), 42.28 (d, C_{-4}), 140.7 (s, C_{-5}), 121.7 (t, C_{-6}), 31.87 (t, C_{-7}), 31.63 (d, C_{-8}), 50.1 (s, C_{-9}), 36.48 (d, C_{-10}), 21.79 (t, C_{-11}), 39.6 (t, C_{-12}), 42.95 (s, C_{-13}), 56.73 (s, C_{-14}) 26.0 (t, C_{-15}), 28.23 (t, C_{-16}), 55.8 (s, C_{-17}), 36.1 (d, C_{-18}), 19.38 (t, C_{-19}), 34.44 (s, C_{-20}), 26.0 (t, C_{-21}), 45.79 (t, C_{-22}), 23.02 (q, C_{-23}), 12.2 (q, C_{-24}), 29.1 (q, C_{-25}), 21.05 (q, C_{-26}), 19.8 (q, C_{-27}), 19.0 (q, C_{-28}), 11.96 (q, C_{-29}).

The results are presented in Table **4.10.**

^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm) : 1.04/1.82 (2H, t, $H_{-1a/b}$), 1.43 (2H, q, $H_{-2a/b}$), 3.5 (1H, m, H_{-3}), 2.23/2.28 (2H, d, H_{-4}), 5.32 (1H, t, H_{-6}), 1.83 (2H, t, H_{-7}), 1.69 (1H, q, H_{-8}), 0.9 (1H, q, H_{-9}), 1.37 (2H, q, H_{-11}), 1.14 (2H, t, H_{-12}), 0.98 (1H, q, H_{-14}), 1.15 (2H, q, H_{-15}), 1.25/1.84 (2H, q, H_{-16}), 1.08 (1H, q, H_{-17}), 1.36 (1H, m, H_{-18}), 0.98 (3H, d, H_{-19}), 1.48 (2H, q, H_{-20}), 1.13 (2H, q, H_{-21}), 1.52 (1H, m, H_{-22}), 1.03 (2H, m, H_{-23}), 0.64 (3H, t, H_{-24}), 1.67 (1H, m, H_{-25}), 0.82 (3H, d, H_{-26}), 1.34, 3H, d, H_{-27}), 0.89 (3H, s, H_{-28}), 0.87 (3H, s, H_{-29}).

The results are presented in Table **4.10.**

FTIR spectrum (V_{max} cm^{-1}, KBr): 3346 (O-H), 2949 (C-H), 1676 (C=C).

3.6.5 Purification of 359TCDE-3 Using Chromatography

TCDE-3 (500 mg) was later subjected to column chromatography on silica gel (70-230 mesh) eluted with 30% stepwise gradient of n-hexane: acetone solvent sysytem. 450 fractions were collected in 20 mL portions. Portion 351-359 (white solid) was labelled 359TCDE-3 (11 mg) and subjected to spectroscopic analysis to characterise.

3.6.6 Characterisation of 359TCDE-3 Using Spectrometry

Appearance: white solid

Melting point: 144.7 – 146.5 ^{o}C

Molecular formula (HREIMS): $C_{28}H_{44}O_3$

Molecular Mass (HREIMS): 428.99 g/mol.

Molecular Mass (calculated): 428.65 g/mol.

Major EIMS m/z fragmentation peaks m/z (relative abundance): 428 (2.2) [M^+], 410 (14), 396 (100), 381 (24), 329 (31), 303 (33), 273 (28), 231 (21), 213 (32), 163 (22), 159 (32), 144 (36), 134 (22), 118 (23), 108 (22), 95 (35), 69 (32), 55 (41).

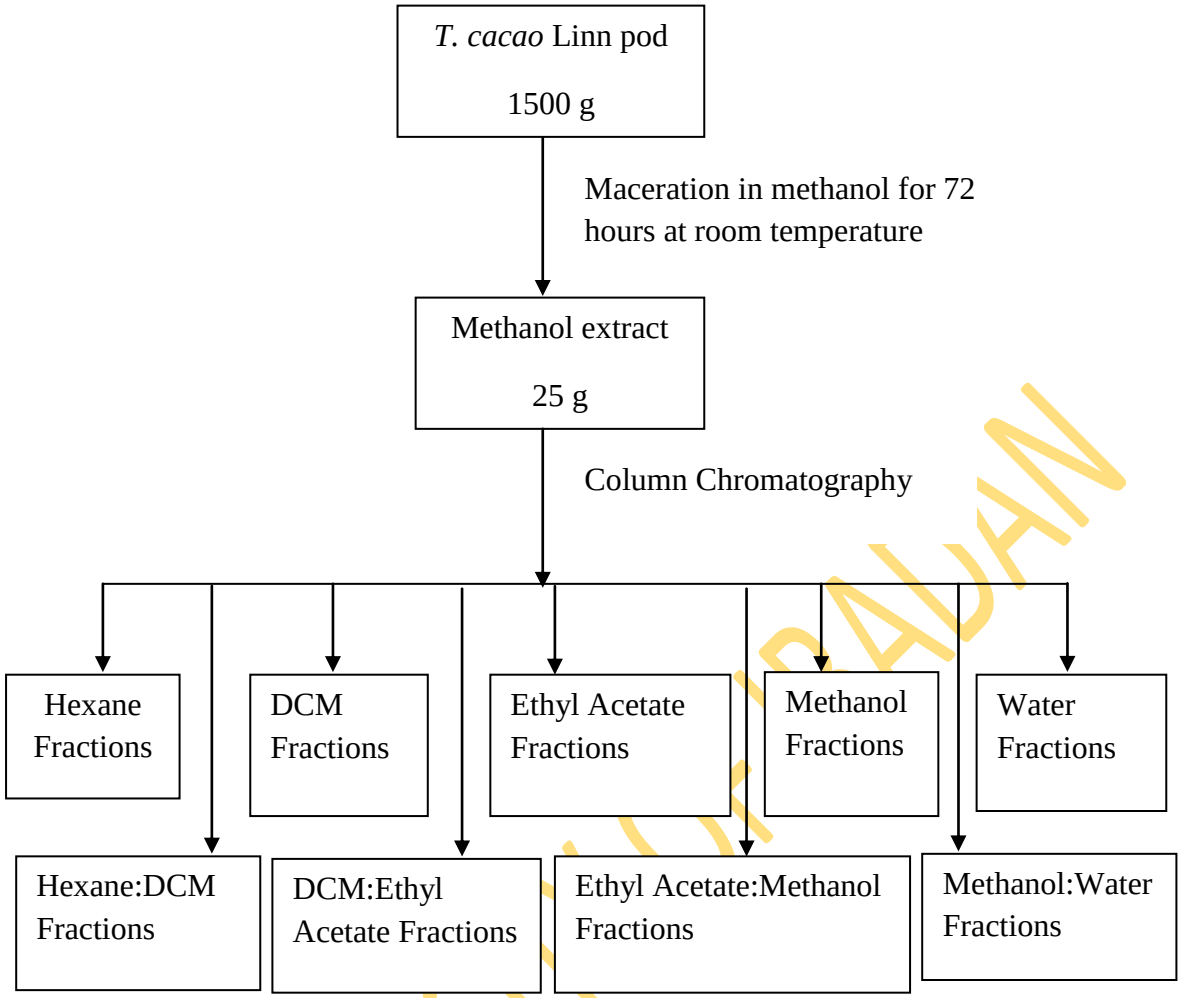
^{13}C-NMR (600 MHz, $CDCl_3$, δ ppm): 34.68 (t, C_{-1}), 30.10 (t, C_{-2}), 66.46 (d, C_{-3}), 36.91 (d, C_{-4}), 79.41 (s, C_{-5}), 130.74 (t, C_{-6}), 135.40 (t, C_{-7}), 82.14 (d, C_{-8}), 51.67 (s, C_{-9}), 36.96 (d, C_{-10}), 20.62 (t, C_{-11}), 39.33 (t, C_{-12}), 44.55 (s, C_{-13}), 51.08 (s, C_{-14}) 23.39 (t, C_{-15}), 28.64 (t, C_{-16}), 56.19 (s, C_{-17}), 12.86 (d, C_{-18}), 18.16 (t, C_{-19}), 39.72 (s, C_{-20}), 20.86 (t, C_{-21}), 135.19 (t, C_{-22}), 132.30 (q, C_{-23}), 42.76 (q, C_{-24}), 33.06 (q, C_{-25}), 19.63 (q, C_{-26}), 19.94 (q, C_{-27}), 17.55 (q, C_{-28}).

The results are presented in Table **4.11.**

^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm) : 1.94/1.68 (2H, t, $H_{-1a/b}$), 1.82/1.51 (2H, q, $H_{-2a/b}$), 3.92 (1H, m, H_{-3}), 2.09/1.91 (2H, d, $H_{-4a/b}$), 6.48 (1H, d, H_{-6}), 6.22 (2H, d, H-$_{7}$), 1.55 (1H, q, H_{-9}), 1.56/1.38 (2H, q, $H_{-11a/b}$), 1.92/1.22 (2H, t, $H_{-12a/b}$), 1.48 (1H, q, H_{-14}), 1.31/1.49 (2H, q, $H_{-15a/b}$), 1.33/1.74 (2H, q, $H_{-16a/b}$), 1.2 (1H, q, H_{-17}), 0.79 (1H, m, H_{-18}), 0.87 (3H, d, H_{-19}), 2.0 (2H, q, H_{-20}), 0.98 (2H, q, H_{-21}), 5.13 (1H, m, H_{-22}), 5.18 (2H, m, H_{-23}), 1.83 (3H, t, H_{-24}), 1.45 (1H, m, H_{-25}), 0.79 (3H, d, H_{-26}), 0.81 (3H, d, H_{-27}), 0.91 (3H, s, H_{-28}).

The results are presented in Table **4.11.**

FTIR spectrum (V_{max} cm^{-1}, KBr): 3346 (O-H), 2954/2875 (C-H), 1676 (C=C).



Scheme 3.2: Extraction and Fractionation Scheme of *T. cacao* Linn Pod-Husk

3.7 Biological Activity of Essential Oils

3.7.1 Antibacterial screening

The essential oils were screened for antibacterial activities against six (6) standard strains of laboratory stock bacteria representing Gram positive and Gram negative (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri* and *Salmonella typhi*). Microplate Alamar Blue Assay was used to determine susceptibility or resistance of the essential oils to selected bacteria strains. Organisms were grown in Mueller Hinton broth and inoculums were adjusted to 0.5 McFarland standard. Stock solutions of the essential oils were prepared in DMSO (1:1 concentration). Media was dispensed to all wells. Essential oils (20 µg/mL) were added in the wells, control wells do not contain essential oil. The volume of 96-well plate was made up to 200 µL. Finally $5x10^6$ cells were added in all wells including both control and test. The plate was sealed with parafilm and incubated for 18 - 20 hours. Alamar Blue Dye was dispensed in each well and shaken at 80 RPM in a shaking incubator for 2 – 3 hours. Plates were covered with foil in shaking incubator. Change in color of Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Absorbance was recorded at 570 nm and 600 nm by the ELISA reader (SpectraMax M2, Molecular Devices, CA, USA). Ampicillin was used as the reference drug. The experiment was done in triplicate.

3.7.2 Antioxidant Activity: DPPH Radical Scavenging Activity

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH using the reported method of Yamaguchi *et al.* (1998). DPPH (*Wako Chemicals* USA, Inc.) solution in methanol was prepared to make 0.3 mM. One milliliter of essential oil (20 µg/mL) was added to 1 mL of the 0.3 mM DPPH solution and shaken vigorously. The reaction is allowed to progress for 30 min at 37 ^oC in the dark and absorbance is monitored by multiplate reader, SpectraMax340, Molecular Devices, CA, USA at 517 nm. Upon reduction, the color of the solution fades (Violet to pale yellow). Absolute methanol was used to zero the spectrophotometer. N-acetylcysteine and gallic acid were used as the reference compounds. The experiment was done in triplicate.

The activity was determined as a function of the % Radical Scavenging Activity which was calculated using the formula;

$$\% \text{ Radical Scavenging Activity} = \left\{\frac{(A_C - A_S}{A_C}\right\} \times 100$$

Where: A_C = Absorbance of the control

A_S = Absorbance of the sample.

3.7.3 Insecticidal Activity

The insecticidal activity was conducted according to the impregnated filter paper method also known as contact toxicity test described by Tabassum *et al.* (1997).

Materials

Test insects (*Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis),* volatile organic solvent (methanol), standard insecticide (Permethrin), petri plates (9 cm diameter), micropipette (1000 µL), growth chamber, test sample, filter paper, glass vials, brush.

Rearing Technique

The stored grain pests were reared in the laboratory under controlled conditions (temperature and humidity) in plastic bottles containing sterile breeding media. Insects of uniform age and size are used for the experiment.

1. **Red flour beetle (*Tribolium castaneum*)**
   Rearing temperature: 30 ^{o}C
   Relative humidity: 50 – 70%
   Rearing media: Wheat flour
   Life cycle: 22-25 days
2. **Lesser grain borer (*Rhyzopertha dominica*)**
   Rearing temperature: 30 ^{o}C
   Relative humidity: 50 – 70%
   Rearing media: Wheat and gram seeds
   Life cycle: 30 days
3. **Pulse beetle (*Callosobruchus analis)***
   Rearing temperature: 25-35 ^{o}C
   Relative humidity: 50 – 70%
   Rearing media: Mung seeds
   Life cycle: 25-30 days

Preparation of Test Sample

Essential oil: 20 µg test sample + 1 mL methanol

Procedure

The filter papers were cut according to the size of glass petri plates (9 cm or 90 mm) and put in the plates. Essential oils were loaded over the filter paper in the plates with the help of micropipette. Ten healthy and active insects of same size and age of each species were put in each plate (test and control) with the help of a clean brush. The plates were incubated at 27 ^{o}C for 24 hours with 50% relative humidity in growth chamber. The survival of the insects was assessed (count the number of survivals of each species).

The Percentage Inhibition or Percentage Mortality was calculated using the formula below:

$$\text{Percentage Mortality} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Test Control

Positive control contained standard insecticide (Permethrin) at the concentration which is effective against all test insects and test insects. Negative control contained volatile solvent (methanol) and the test insects.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Essential Oils

The volatile extracts/essential oils (EOs) of the twenty-seven (27) samples were obtained by the process of hydrodistillation using an all-glass Clevenger apparatus. The physicochemical properties of the essential oils were determined and the EOs were analysed by Gas Chromatography (Flame Ionization Detector and Mass Spectrometry) techniques to determine their chemical constituents. The oils were also investigated for their antibacterial, antioxidant and insecticidal activities.

4.1.1 Essential Oils Yield

The physicochemical properties of the essential oils are shown in Table **4.1**. The characteristic colours of the oils ranged from colourless to pale yellow with yields ranging between 0.13 and 1.21% v/w. All the fruits have sweet aromatic and fruity smell reported to be due to the presence of esters, aldehydes, alcohols, terpenes or their derivatives, but the oils of the root bark and stem barks have irritating woody smell while the leaves had a stong leafy odour. *T. cacao* seed had a malty odour.

Table 4.1: Physicochemical Properties of Essential Oils

S/N	Plant Sample	Part used	% Extract Composition	Colour	Odour
1	*Persea americana*	Stem Bark	0.8	Colourless	Herbal
		Root Bark	0.72	Colourless	Herbal
		Leaf	0.71	Colourless	Leafy
		Peel	0.51	Colourless	Irritating
		Fruit	0.23	Pale Yellow	Leafy
		Seed	0.47	Colourless	Irritating
2	*Carica papaya*	Root Bark	0.46	Colourless	Woody
		Root	0.26	Colourless	Woody
		Stem Bark	0.47	Colourless	Woody
		Stem	0.21	Colourless	Woody
		Leaf	0.89	Pale Yellow	Leafy
		Fruit	0.29	Pale Yellow	Fruity
		Seed	0.91	Colourless	Sweet Aromatic
		Peel	0.49	Pale Yellow	Fruity
3	*Ananas comosus*	Shoot	0.48	Colourless	Leafy
		Peel	0.71	Colourless	Fruity
		Fruit	0.62	Colourless	Fruity
4	*Theobroma cacao*	Stem Bark	1.29	Colourless	Herbal
		Leaf	1.03	Colourless	Leafy
		Pod	0.65	Colourless	Irritating
		Seed	1.15	Pale yellow	Malty
5	*Chrysophyllum albidum*	Stem Bark	0.86	Colourless	Woody
		Root Bark	1.21	Colourless	Woody
		Leaf	0.89	Pale Yellow	Leafy
		Seed	0.91	Colourless	Sweet aromatic
		Seed Bark	0.13	Colourless	Herbal
		Fruit Bark	0.95	Pale Yellow	Fruity

4.1.2 Chemical Composition of Essential Oils

4.1.2.1 *Persea americana* M.

The chemical and percentage compositions of the essential oils of the plant parts of *Persea americana* from the GC chromatograms (Figures **4.1**-**4.6**) revealed a total of 13 to 48 constituents representing 83.09 to 96.58% composition (Table **4.2**). The major components in the fruit oil were p-xylene (40.51%), trans-nerolidol (16.17%), heptacosane (12.96%), and 2,2[1]-methylenebis tertbutyl-4-ethylphenol (5.48%). The essential oil from the peel was dominated by globulol (25.43%), trans-nerolidol (17.04%), β-elemene (13.62%) and hexadecanoic acid (13.42%). The prevalence of (Z,Z)- 4,15-octadecadien-1-ol acetate (21.77%), β-elemene (15.54%) and p-xylene (7.67%) were observed in the seed oil while leaf oil had β-caryophyllene (12.67%), trans-phytol (11.81%), p-xylene (5.22%) and δ-cadinene (3.85%) as the major compounds. Tetradecanal (24.99%), dodecanal (9.43%, tridecanal (7.32%) and β-caryophyllene oxide (4.59%) were the major compounds in the stem bark oil while tetradecanal (31.84%), dodecanal (13.17%) and tetradecanoic acid (7.25%) dominated the root bark oil.

Furthermore, on analysis of the class of compounds present, the essential oils from the peel and leaf were dominated by sesquiterpenes (68.37% and 40.79% respectively) while the other oils had more non-terpenes (71.4% to 78.65%). Apocarotenes and diterpenes were absent in all the oils except the leaf oil with 2.09% and 11.81% composition, respectively. Triterpene (squalene- 0.55%) was present in only the seed oil. Monoterpenes were present in all the oils except the peel oil. The percentage compositions of the monoterpenes were low (0.11% to 11.08%).

The result obtained from this analysis compare favourably with respect to the predominance of β-caryophyllene in the leaf oil as reported in previous research by Ogunbinu *et al.* (2007). Larijani *et al.* (2010) however reported methyl eugenol as the major compound while β-caryophyllene was the second predominant compound. Sinyinda and Gramshaw (1998) also found β-caryophyllene to be the major constituent in the mesocarp (fruit) essential oil.

The compositional pattern reveals the presence of β-elemene at an appreciable quantity in the peel and seed oil, trans-nerolidol was relatively dominant in the fruit and peel

oil. The stem bark and root bark oils were dominated by aldehydes (tetradecanal, dodecanal and tridecanal). Phytol was found to be present in only the leaf oil and the percentage composition was relatively high (11.81%) making it the second dominant compound. It is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K 1 (Daines *et al.*, 2003; Igwe, 2014). Phytol is used in the fragrance industry and in cosmetics, shampoos, toilet soaps, household cleaners and detergents (McGinty, 2010; Igwe, 2014). Application of phytol also include infection fighting and natural alternativetherapies for hypertension and cancer (Daines *et al.*, 2003; McGinty, 2010; Igwe, 2014). Phytol has been reported to have anti-mycobacterial activity against *Mycobacterium tuberculosis* (Rajab, 1998; Daines *et al.*, 2003; Igwe, 2014). The high quantity of phytol in the leaf of *P. americana* suggests that the plant might be used in the treatment of tuberculosis.

This study represents the first comprehensive characterization of the volatile constituents of the essential oil of all the plant parts of *Persea americana* Mill grown in Nigeria.

Table 4.2: Essential Oil Components of *Persea Americana* Mill

S/N	Compound Name	RI	PAF	PAP	PASE	PAL	PASB	PARB
1	Ethylbenzene	893	1.77	-	1.89	1.29	0.51	-
2	(+)Sabinene	897	-	-	-	2.77	-	-
3	**p-Xylene**	**907**	**40.51**	0.56	**7.67**	**5.22**	0.59	-
4	m-Xylene	907	-	-	-	-	2.14	-
5	(-)β-Pinene	943	-	-	0.52	2.67	-	-
6	Camphene	943	-	-	0.38	-	-	0.79
7	1R-α-Pinene	948	0.32	-	0.88	2.55	-	0.38
8	S-3-Carene	948	-	-	-	0.21	0.11	-
9	**Irid-2-ene**	**957**	-	-	**3.15**	-	-	-
10	m-Ethyltoluene	1006	0.54	-	0.75	-	-	-
11	Decane	1015	0.54	-	0.26	0.26	0.21	-
12	D-Limonene	1018	0.87	-	0.23	0.38	-	-
13	Hemimelitene	1020	0.61	-	-	-	-	-
14	α-Terpinolene	1052	0.86	-	-	-	-	-
15	1,8-Cineole	1059	-	-	-	1.28	-	-
16	β-Linalol	1082	-	-	-	0.21	-	-
17	Camphene Hydrate	1088	-	-	-	-	-	0.36
18	Borneol	1088	-	-	-	-	-	0.49
19	Nonanal	1104	-	0.66	0.52	1.39	0.18	-
20	Undecane	1115	1.79	-	-	-	0.11	-
21	4-Terpineol	1137	-	-	-	0.51	-	-
22	Exo-Fenchol	1138	-	-	-	-	-	0.34
23	(-)Cis Myrtanol	1180	-	-	-	0.29	-	-
24	Methyl Nonanoate	1183	-	-	-	-	0.13	-
25	β-Cyclocitral	1204	-	-	-	0.21	-	-
26	Decanal	1204	-	-	-	-	0.29	0.23
27	Cis-7-Decen-1-ol	1212	-	-	0.53	-	-	-
28	(-)α-Copaene	1221	-	-	0.24	2.35	-	-
29	9-Decen-1-ol	1248	-	-	0.44	-	-	-
30	4,6-Dimethyldodecane	1285	0.58	-	-	-	-	-
31	10-Undecanal	1293	-	-	3.14	-	-	3.09
32	Ledene Oxide	1293	-	-	-	-	3.74	-
33	α-Cedrene Epoxide	1293	-	-	-	-	2.07	-
34	**Undecanal**	**1303**	-	-	0.26	-	2.89	**5.07**
35	Tridecane	1313	3.43	-	-	-	-	-
36	2,3-Tridecene	1321	0.44	-	-	-	-	-
37	2-Octylfuran	1338	-	-	1.09	1.04	-	-
38	11-Dodecen-2-one	1340	-	-	0.89	-	-	0.39
39	(-)α-Cubebene	1344	-	-	-	1.53	-	-

Table 4.2 continued

S/N	Compound Name	RI	PAF	PAP	PASE	PAL	PASB	PARB
40	**10-Undecen-1-ol**	**1347**	**-**	**-**	**-**	**-**	**-**	**4.65**
41	10-Undecyn-1-ol	1355	-	-	0.49	-	-	0.54
42	Undecanol	1357	-	-	-	-	3.41	-
43	2-Butyl octanol	1393	0.41	-	-	-	-	-
44	**(-)β-Elemene**	**1398**	**-**	**13.62**	**15.54**	1.54	-	1.31
45	**Dodecanal**	**1402**	**-**	**-**	0.34	0.37	**9.43**	**13.17**
46	α-Cedrene	1403	-	-	-	0.28	-	-
47	Tetradecane	1413	0.52	-	-	-	-	-
48	α-Ionone	1429	-	-	-	0.85	-	-
49	α-Bergamotene	1430	-	-	-	0.91	-	-
50	γ-Muurolene	1435	-	-	-	1.08	-	-
51	β-Farnesene	1440	0.78	-	-	-	-	-
52	2-Tridecanone	1449	-	-	-	-	-	0.74
53	γ-Elemene	1465	-	-	-	1.37	-	-
54	β-Selinene	1469	-	-	-	0.74	-	-
55	**(-)δ-Cadinene**	**1469**	**-**	**-**	**-**	**3.85**	-	-
56	α-Selinene	1474	0.61	1.82	-	1.21	-	-
57	Acoradiene	1474	-	-	-	0.21	-	-
58	**β-Caryophyllene**	**1494**	-	1.31	1.21	**12.67**	-	-
59	(+)β-Bisabolene	1500	-	-	-	1.71	-	-
60	**Tridecanal**	**1502**	-	-	1.32	-	**7.32**	2.36
61	**β-Caryophyllene Epoxide**	**1507**	0.62	-	**2.42**	0.77	**4.59**	-
62	Trans-2- Tridecenal	1510	-	-	0.42	-	-	-
63	Pentadecane	1512	0.53	-	-	-	-	-
64	(+)Ledol	1530	-	-	-	0.79	-	-
65	**Globulol**	**1530**	**-**	**25.43**	-	-	-	0.81
66	Trans-α -Bisabolene Epoxide	1531	-	-	-	-	0.94	-
67	(-)Calamenene	1537	-	-	-	-	2.36	0.57
68	2-Tetradecanone	1549	-	-	0.72	-	-	-
69	**Tridecanol**	**1556**	**-**	**-**	**-**	**-**	**6.52**	-
70	γ-Gurjunene Epoxide	1558	-	-	-	-	-	1.42
71	**(±)Trans Nerolidol**	**1564**	**16.17**	**17.04**	0.71	0.67	-	-
72	**Dodecanoic Acid**	**1570**	**-**	**-**	**-**	**-**	1.41	**4.31**
73	**11-Tridecyn-1-ol**	**1574**	**-**	**-**	**-**	**-**	**-**	**4.66**
74	α-Caryophyllene	1579	-	0.57	-	2.65	-	-
75	Cubenol	1580	-	2.91	-	1.81	1.73	-
76	**Tau-Muurolol**	**1580**	**-**	**-**	**-**	0.61	**3.33**	-
77	**α-Cadinol**	**1580**	**-**	**-**	**-**	0.41	**2.58**	-
78	(-)δ-Cadinol	1580	-	-	-	-	0.89	-
79	**13-Tetradecenal**	**1591**	**-**	**-**	1.35	**-**	**-**	**19.19**

Table 4.2 continued

S/N	Compound Name	RI	PAF	PAP	PASE	PAL	PASB	PARB
80	**Tetradecanal**	**1601**	-	-	1.05	0.89	**24.99**	**12.65**
81	Dendrolasin	1607	-	-	-	1.71	-	-
82	Cis-9-Tetradecenol	1609	-	-	0.65	-	0.71	-
83	β-Bisabolol	1619	-	-	-	1.43	-	-
84	α-Bisabolol	1625	-	-	-	0.49	-	-
85	**Germacrene-D-4-ol**	**1660**	-	**4.81**	-	-	-	-
86	13-Tetradece-11-yn-1-ol	1663	-	-	0.29	-	-	-
87	Tridecanoic Acid	1670	-	-	-	-	3.47	-
88	Trans-α-Bergamotol	1673	-	0.86	-	-	-	-
89	Pentadecanal	1701	-	-	-	0.38	-	-
90	Cyclododecyl Ethanone	1735	-	-	0.79	-	-	-
91	Hexahydrofarnesyl acetone	1754	-	-	-	0.43	-	-
92	Humulane-1,6-dien-3-ol	1757	0.59	-	-	-	-	-
93	**Tetradecanoic Acid**	**1767**	-	-	-	-	-	**7.25**
94	Tetradecanoate	1779	-	-	-	0.75	-	-
95	1,2,15,16-Diepoxyhexadecane	1792	-	-	0.38	-	-	-
96	Cis-7-Hexadecenal	1808	-	3.56	-	-	0.42	-
97	Cis-9-Hexadecanal	1808	-	-	-	-	0.56	-
98	Cis-7,10-Hexadecadienal	1816	-	-	0.59	-	-	-
99	Pentadecanoic Acid	1869	-	-	-	-	3.32	-
100	Farnesyl acetone	1902	-	-	-	0.81	-	-
101	Nonadecane	1910	4.61	-	-	-	-	-
102	Heptadecanol	1954	-	-	-	3.92	-	-
103	**Hexadecanoic Acid**	**1968**	-	**13.42**	0.57	-	2.79	-
104	Cyclopentadecanone	1970	-	-	0.79	-	-	-
105	Cis-9,17-Octadecadienal	1997	-	-	0.52	-	-	-
106	Sulforous acid, nonyl pentyl ester	2036	0.39	-	-	-	-	-
107	**Trans Phytol**	**2045**	-	-	-	**11.81**	-	-
108	8-Cyclohexadecen-1-one	2072	-	-	0.23	-	-	-
109	**Bicyclo [10,6,0]Octadeca-1(12),15-diene**	**2082**	-	-	**7.32**	-	-	-
110	17-Octadecynoic Acid	2165	0.65	-	-	-	-	0.35
111	Cis-9-Octadecenoic Acid	2175	-	-	0.23	-	-	-
112	**Cis-4,15-Octadecadien-1-ol acetate**	**2193**	-	-	**21.77**	-	-	-
113	**Heptacosane**	**2705**	**12.96**	-	-	**2.31**	-	-
114	Squalene	2914	-	-	0.55	-	-	-
115	**2,2[1]-Methylenebis(6-tertbutyl-4-ethyl)Phenol**	**2987**	**5.48**	-	-	-	-	-
	Total		96.58	86.57	83.09	83.59	93.74	85.12
	No. of Compounds		25	13	40	48	31	23

Table 4.2 continued

S/N	Compound Name	RI	PAF	PAP	PASE	PAL	PASB	PARB
	Monoterpenes		2.05	-	5.16	11.08	0.11	2.36
	Sesquiterpenes		18.77	68.37	4.58	40.79	22.23	4.11
	Diterpenes		-	-	-	11.81	-	-
	Triterpenes		-	-	0.55	-	-	-
	Apocarotenes		-	-	-	2.09	-	-
	Non-terpenes		75.76	18.2	72.8	17.82	71.4	78.65

Key: PAF- *Persea americana* Fruit, PAP- *Persea americana* Peel,
PASE- *Persea americana* Seed, PAL- *Persea americana* Leaf,
PASB- *Persea americana* Stem Bark, PARB- *Persea americana* Root Bark
RI-Retention Index

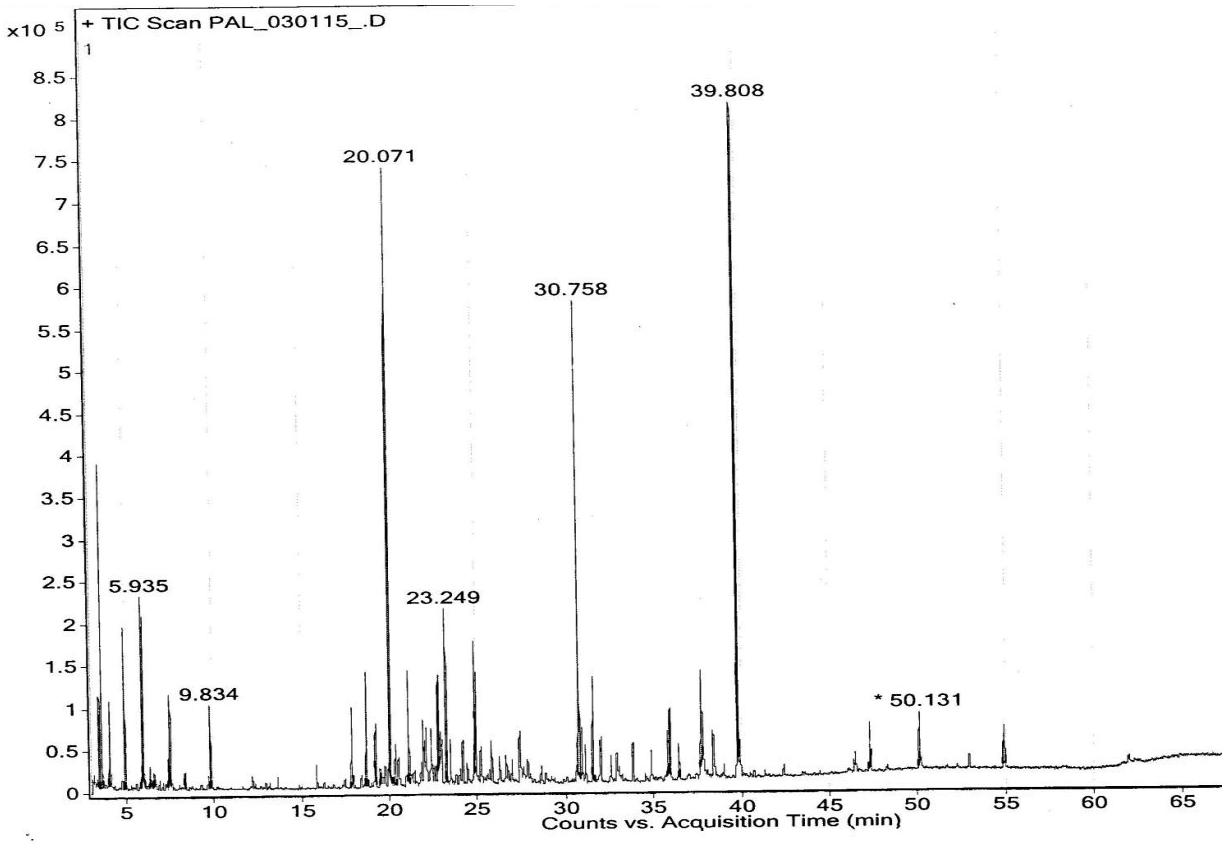


Figure 4.1: GC Chromatogram of *Persea americana* Leaf

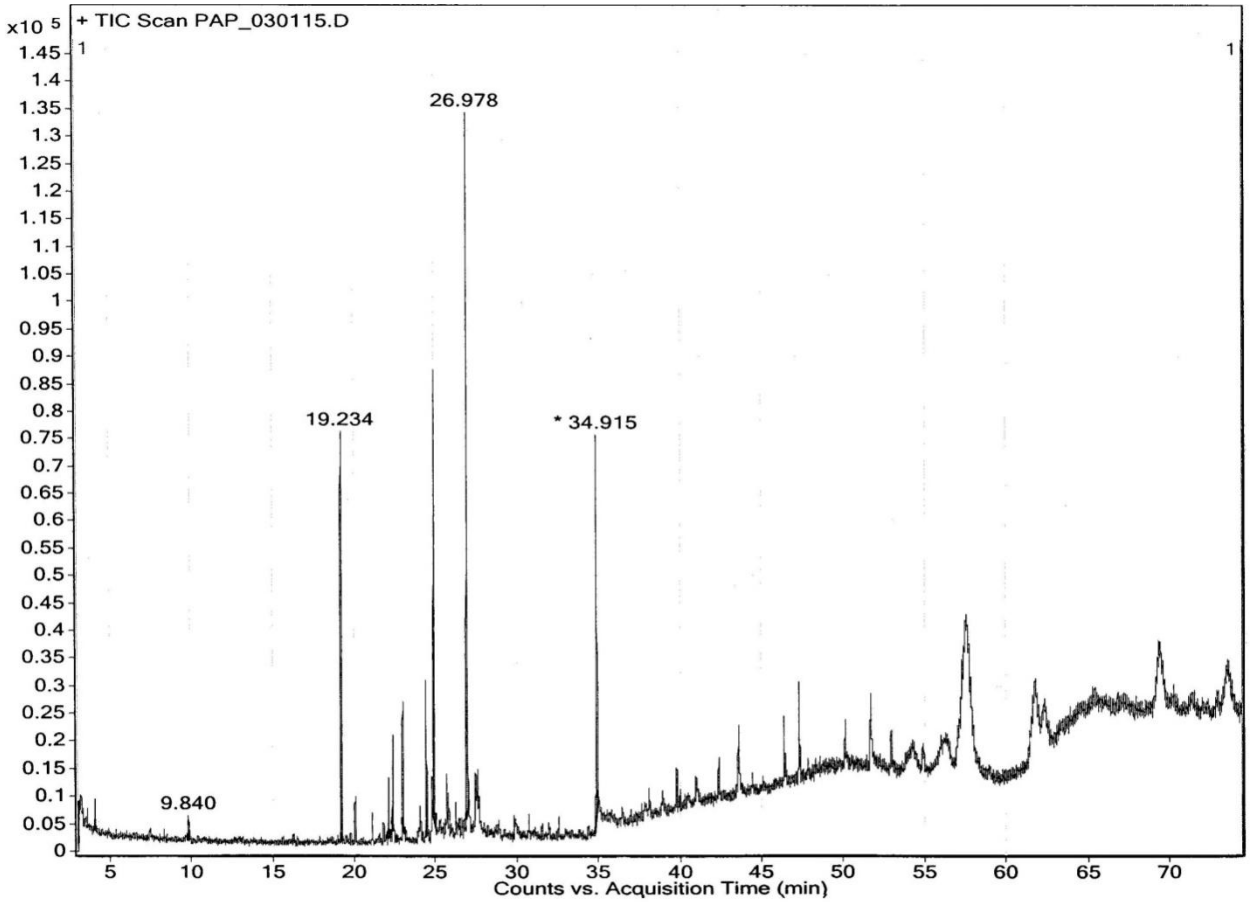


Figure 4.2: GC Chromatogram of *Persea americana* Peel

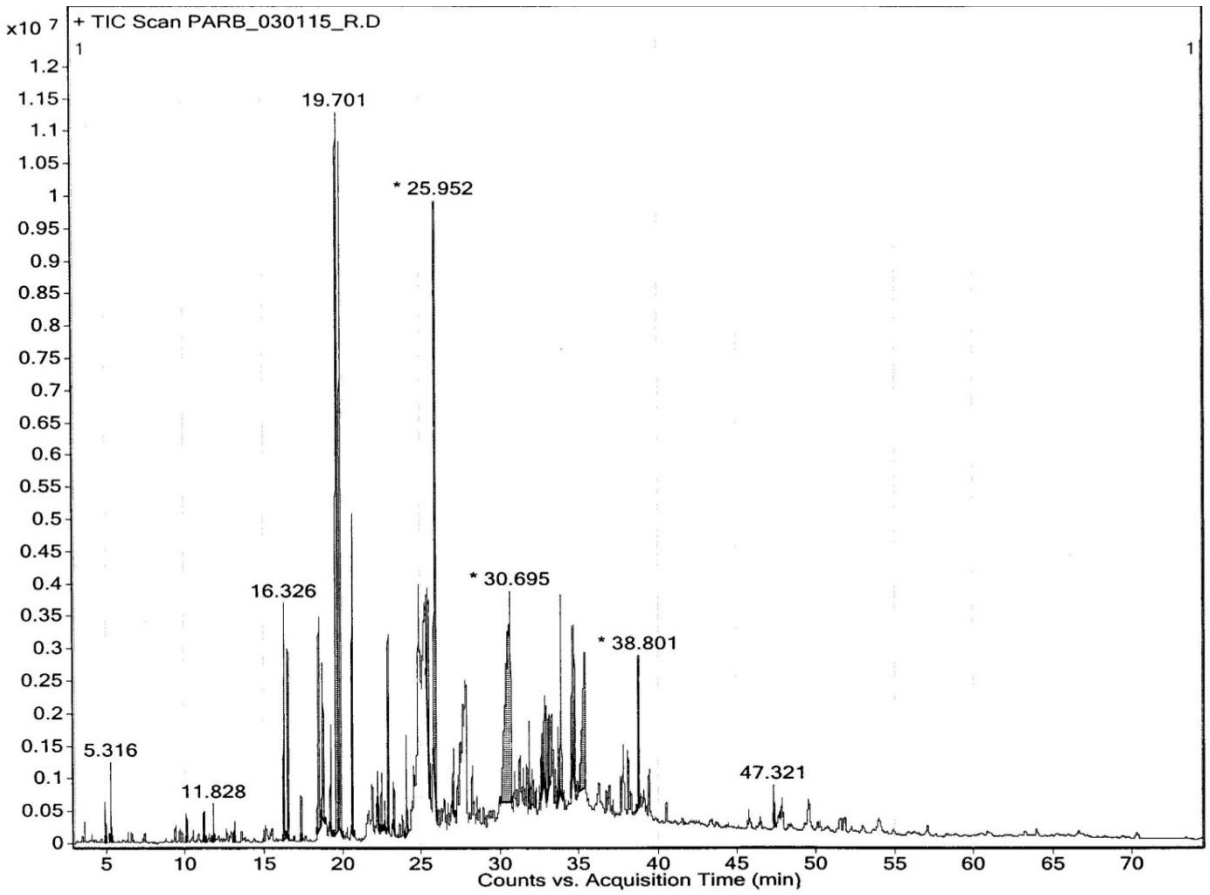


Figure 4.3: GC Chromatogram of *Persea americana* Root Bark

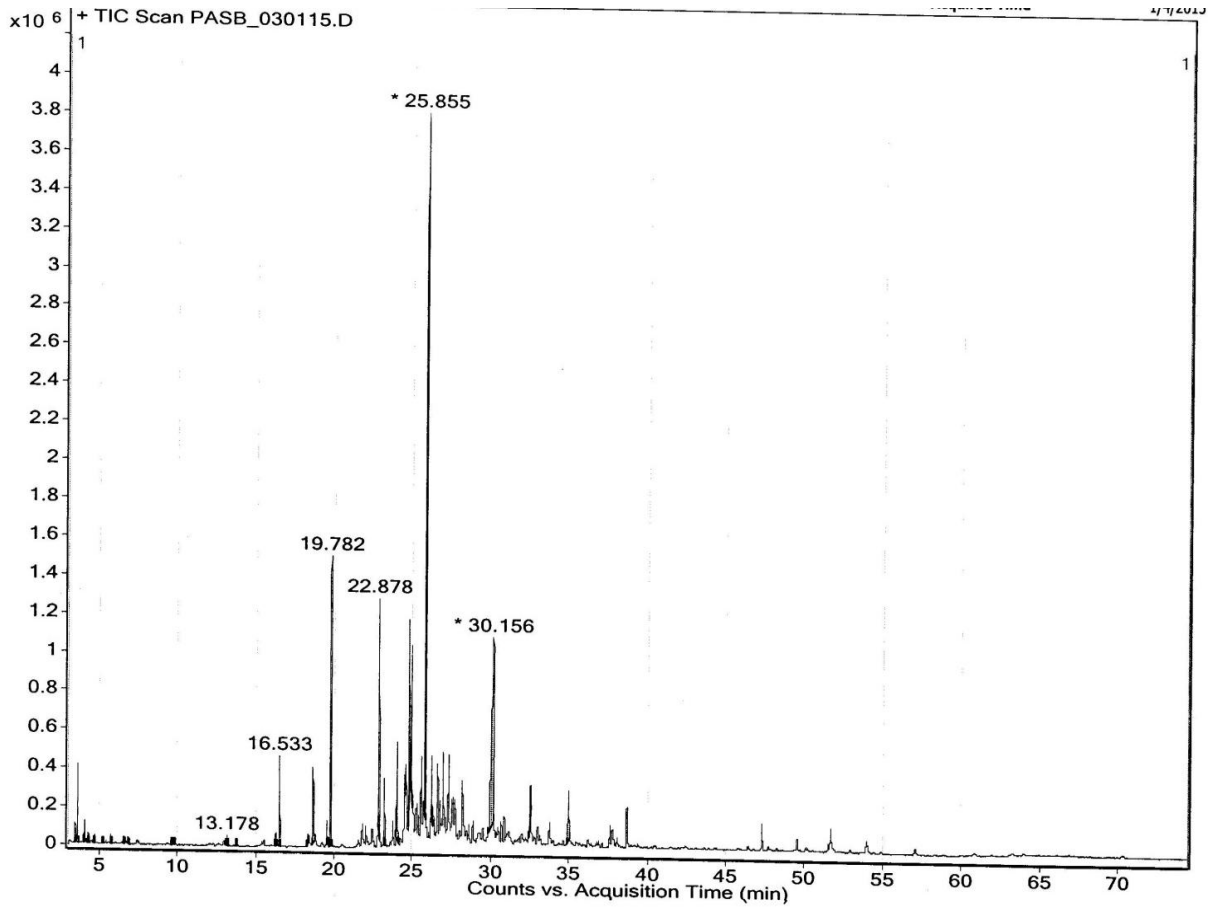


Figure 4.4: GC Chromatogram of *Persea americana* Stem Bark

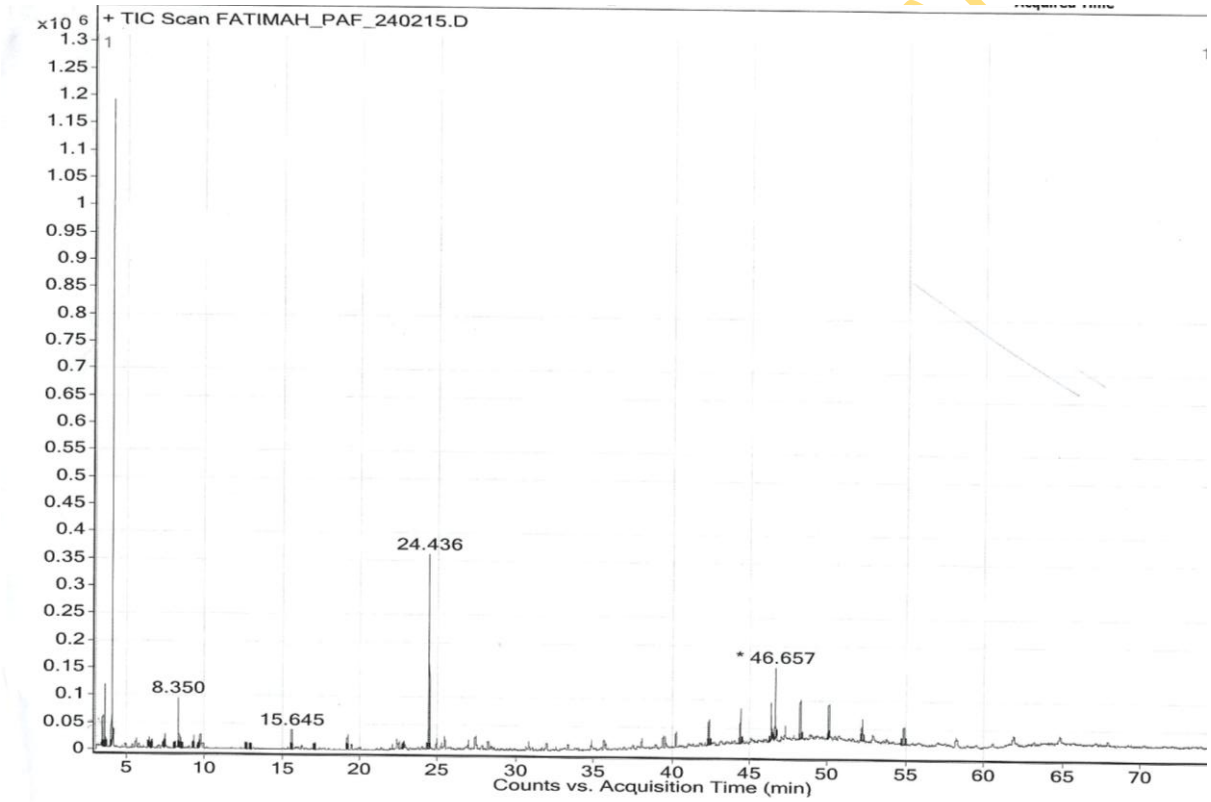


Figure 4.5: GC Chromatogram of *Persea americana* Fruit

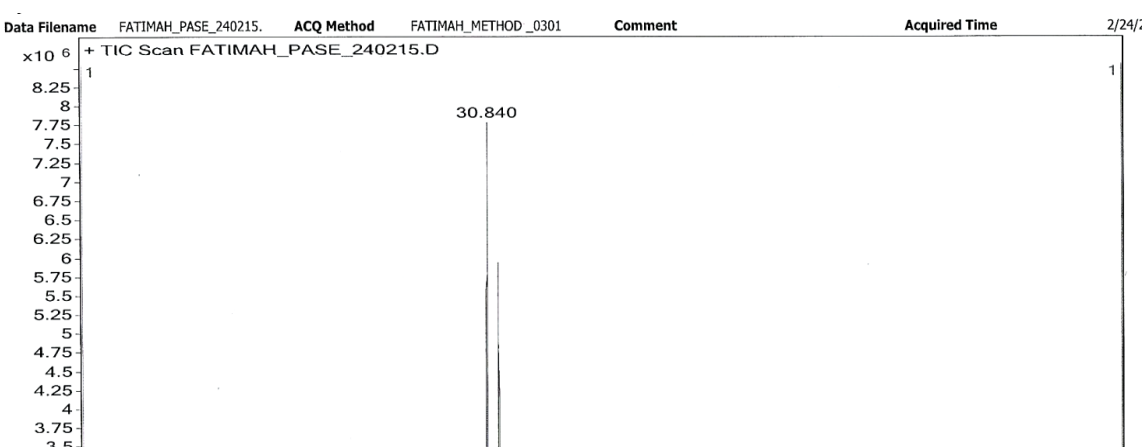


Figure 4.6: GC Chromatogram of *Persea americana* Seed

4.1.2.2 *Carica papaya*

The GC (Figures **4.7-4.14**) and GCMS analyses of essential oils from *Carica papaya* fruit, leaf, peel, root bark, root, stem bark, stem and seed afforded 46, 40, 23, 26, 10, 16, 11 and 9 compounds identified, representing 94.32%, 90.19%, 72.03%, 94.67%, 97.06%, 98.11%, 93.01% and 99.58% of essential oil composition, respectively (Table **4.3**). The dominant constituents in the fruit oil are heptadecanol (25.17%), phytol

(10.64%) and hexadecanoic acid (8.44%). The leaf oil has phytol (21.83%), farnesyl acetone (10.69%) and heptacosane (10.59%) as its major compounds while 9-hexadecen-1-ol (16.97%), m-xylene (14.5%), heptacosane (7.58%) and squalene (6.57%) were predominant amongst the identified compounds in the peel oil. Benzylisothiocyanate (71.54%) and octadecanol (62.54%) were the major constituents in the essential oil of the root bark and the root respectively. m-xylene (35.11%), heptacosane (22.01%), p-xylene (12.46%) and ethylbenzene (10.48%) dominate the compounds identified in the essential oil from the stem bark while the stem oil was dominated by octadecanol (71.13%), hexadecanol (6.31%) and heptacosane (6.23%). The seed oil however has benzylisothiocyanate (89.12%) as the major constituent.

Analyses of the chemical constituents based on the class of compounds revealed the presence of monoterpenes, though in low quantity, in all the oils except the leaf, stem and seed oils. The fruit and leaf oil have 10.64% and 22.16% diterpenes, respectively while triterpenes were present in all the oils except root and seed oils. The oils had high percentage of non-terpene compounds within the range of 54.65% to 99.43% except for the leaf oil with 26.41%. Four, five and one apocarotene compounds representing 15.75%, 27.8% and 1.24% were identified in the fruit, leaf and peel oil, respectively.

In an earlier study on the volatile constituents of *Carica papaya* leaf of Nigerian origin, six compounds were identified with phytol (37.78%), 9-octadecenamide (28.18%), cyclopentaneundecanoic acid methyl ester (12.02%) and 3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide (11.78%) as the major constituents (Igwe, 2015). However, in the present study, more constituents were identified. Hexahydrofarnesyl acetone (7.77%), geranyl acetone (5.96%), squalene (3.62%) and benzylisothiocyanate (3.03%) were identified in appreciable quantity in the leaf oil of this study but were not reported in the earlier study by Igwe (2015) although phytol was found to be the major and only common compound in both studies. The presence of benzylisothiocyanate which is an organosulphur compound (OSC) in the oils of the fruit, leaf, root bark, root and seed is a probable indication that the oils will have anticarcinogenic property. Organosulphur compounds (OSCs) prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens. OSCs offer protection against cancer (Igwe, 2015). These include

inhibition of the carcinogens, dermatitis and other minor wounds (Okwu and Ighodoro, 2009). OSCs have also been reported to have numerous beneficial health effects including protection from oxidative damage (Dwivedi *et al.*, 1998; Siegers *et al.*, 1999; Igwe and Okwu, 2013).

The compositional patterns of the volatile constituents of the other parts of *Carica papaya* plant of Nigerian origin are reported here for the first time.

Table 4.3: Volatile Constituents of *Carica papaya* Plant Parts

S/N	Compound Name	RI	CPF	CPL	CPP	CPRB	CPR	CPSB	CPS	CPSE
1	4-Methyl Octane	852	-	1.79	0.22	0.08	-	-	-	-
2	**Ethylbenzene**	**893**	0.63	-	-	-	2.65	**10.48**	0.22	-
3	**p-Xylene**	**907**	2.49	-	-	-	**15.43**	**12.46**	1.38	-

4	**m-Xylene**	**907**	**-**	**-**	**14.5**	**4.91**	**-**	**35.11**	**-**	**-**
5	o-Xylene	907	-	-	0.33	0.09	-	-	-	-
6	Nonane	916	-	-	-	-	-	0.85	-	-
7	Cumene	928	-	-	-	-	-	0.47	-	-
8	6-Methyl-5-hepta-2-one	938	0.61	-	-	-	-	-	-	-
9	(+)2-Carene	948	-	-	-	0.13	-	-	-	-
10	1-Octen-3-ol	969	0.77	-	-	0.13	-	-	-	-
11	Benzaldehyde	982	-	-	-	-	-	-	-	0.69
12	o-Ethyltoluene	1006	-	-	-	-	1.49	0.39	-	-
13	Decane	1015	0.47	-	-	-	0.65	1.78	-	-
14	D-Limonene	1018	-	-	0.33	0.31	-	1.04	-	-
15	Hemimelitene	1020	-	-	-	-	0.97	2.16	-	-
16	2-Pentylfuran	1040	0.35	-	-	-	-	-	-	-
17	α-Terpinolene	1052	-	-	0.39	-	-	0.66	-	-
18	1,8-Cineole	1059	-	-	-	-	1.94	-	-	-
19	β-Linalol	1082	0.28	-	-	-	-	-	-	-
20	Nonanal	1104	1.69	0.69	-	0.23	-	-	0.11	-
21	Undecane	1115	-	-	0.33	-	-	-	-	-
22	2,4,6-Trimethyldecane	1121	-	-	-	0.49	-	-	-	-
23	Trans-3(10)-Caren-2-ol	1131	0.15	-	-	-	-	-	-	-
24	3-Caren-10-al	1136	0.21	-	-	-	-	-	-	-
25	**Benzene Acetonitrile**	**1138**	1.24	-	-	1.78	2.19	-	-	**6.96**
26	2-Methyl undecane	1150	-	-	-	0.19	-	-	-	-
27	Methyl nonanoate	1183	-	-	-	0.23	-	2.16	-	-
28	β-Cyclocitral	1204	0.24	-	-	-	-	-	-	-
29	Decanal	1204	-	0.28	0.33	0.17	-	-	-	-
30	Trans-2,4-Decadienal	1220	-	-	-	0.15	-	-	-	-
31	(+)α-Copaene	1221	0.77	-	-	-	-	-	-	-
32	(-)α-Copaene	1221	-	0.58	-	-	-	-	-	-
33	**Benzylisothiocyanate**	**1318**	**2.2**	**3.03**	**-**	**71.54**	**2.14**	**-**	**-**	**89.12**
34	Farnesane	1320	-	-	2.02	-	-	-	-	-
35	(+)Aromadendrene	1386	0.28	0.36	-	-	-	-	-	-
36	(-)β-Elemene	1398	0.51	0.71	-	-	-	-	-	0.15
37	α-Cedrene	1403	0.31	-	-	-	-	-	-	-
38	β-Gurjunene	1403	0.66	0.67	-	-	-	-	-	-

Table 4.3 continued

S/N	Compound Name	RI	CPF	CPL	CPP	CPRB	CPR	CPSB	CPS	CPSE
39	Tetradecane	1413	-	-	0.37	-	-	0.52	-	-
40	(+)Ledene	1419	0.53	0.49	-	-	-	-	-	-
41	**Geranyl Acetone**	**1420**	**4.18**	**5.96**	-	0.12	-	-	-	-
42	Trans-ionone	1428	0.21	0.55	-	-	-	-	-	-

43	α-Ionone	1429	0.53	0.57	-	-	-	-	-	-
44	α-Bergamotene	1430	-	0.22	-	-	-	-	-	-
45	α-Muurolene	1440	0.36	0.29	-	-	-	-	-	-
46	β-Ionone	1457	1.33	1.81	-	-	-	-	-	-
47	(+)δ-Cadinene	1469	1.99	1.58	-	-	-	-	-	-
48	Acoradiene	1474	0.45	0.56	-	-	-	-	-	-
49	β-Caryophyllene	1494	0.51	1.52	-	-	-	-	-	-
50	(±)β-Bisabolene	1500	0.18	0.26	-	-	-	-	-	-
51	Caryophyllene Epoxide	1507	0.32	0.56	-	0.18	-	-	-	-
52	2,6,10-Trimethyltetradecane	1519	0.39	-	-	0.92	-	1.49	-	-
53	α-Curcumene	1524	0.64	0.71	-	-	-	-	-	-
54	(+)Ledol	1530	0.31	-	-	0.42	-	-	-	-
55	Epiglobulol	1530	0.22	-	-	-	-	-	-	-
56	Globulol	1530	-	0.68	-	-	-	-	-	-
57	Hexahydrofarnesol	1563	-	0.45	-	-	-	-	-	-
58	(±)Trans Nerolidol	1564	1.55	0.73	-	0.13	-	-	-	-
59	α-Cadinol	1580	-	0.28	-	-	-	-	-	-
60	Tetradecanal	1601	-	0.86	-	-	-	-	-	0.09
61	Cis-7-Hexadecane	1620	-	-	0.29	-	-	-	-	-
62	Trans-2-Tetradecen-1-ol	1664	-	0.36	-	-	-	-	-	-
63	Hexadecylene oxide	1702	-	-	-	0.26	-	-	-	-
64	**Hexahydrofarnesyl Acetone**	**1754**	**3.74**	**7.77**	1.24	-	-	-	-	-
65	Humulane-1,6-dien-3-ol	1757	-	-	-	-	-	-	0.47	-
66	Cis-6-Pentadecen-1-ol	1763	-	-	**3.65**	-	-	-	-	-
67	Tetradecanoic Acid	1769	**3.43**	-	-	0.45	-	-	-	-
68	2-Hexyl-1-decanol	1790	-	-	0.96	-	-	-	-	-
69	1,2,15,16-Diepoxyhexadecane	1792	0.45	-	-	-	-	-	-	-
70	Isopropyl Myristate	1814	0.29	-	-	-	-	-	-	-
71	**Hexadecanol**	**1854**	**5.01**	-	0.36	-	**7.06**	-	**6.31**	-
72	**9-Hexadecen-1-ol**	**1862**	-	-	**16.97**	-	-	-	-	-
73	Methyl Hexadecanoate	1878	0.26	0.55	-	-	-	-	-	0.19
74	Isophytol	1899	-	0.33	-	-	-	-	-	-
75	Nonadecene	1900	-	-	4.05	-	-	-	-	-

Table 4.3 continued

S/N	Compound Name	RI	CPF	CPL	CPP	CPRB	CPR	CPSB	CPS	CPSE
76	**Farnesyl Acetone**	**1902**	**5.76**	**10.69**	-	-	-	-	-	-
77	Nonadecane	1910	-	-	1.09	-	-	-	1.45	-
78	**Heptadecanol**	**1954**	**25.17**	**3.51**	-	-	-	-	-	1.51
79	**Hexadecanoic Acid**	**1968**	**8.44**	1.21	**6.42**	**5.99**	-	-	0.37	-

80	γ-Palmito acetone	1980	-	-	-	0.63	-	-	-	-
81	**Trans Phytol**	**2045**	**10.64**	**21.83**	-	-	-	-	-	-
82	**Octadecanol**	**2053**	**-**	**-**	**-**	**-**	**62.54**	**-**	**71.13**	**-**
83	Cis-9,12-Octadecadien-1-ol	2069	-	-	3.25	-	-	-	-	-
84	Methyl trans-9-octadecanoate	2085	-	-	-	-	-	-	-	0.51
85	Ethyl-9-octadecanoate	2185	-	-	-	-	-	-	-	0.36
86	Eicosanol	2252	-	-	-	2.17	-	-	-	-
87	Docosanol	2451	-	0.28	-	-	-	-	-	-
88	**Heptacosane**	**2705**	0.76	**10.59**	**7.58**	2.19	**-**	**22.01**	**6.23**	-
89	α-Glyceryl Linolenate	2705	-	0.64	-	-	-	-	-	-
90	**Squalene**	**2914**	2.81	**3.62**	**6.57**	0.78	**-**	1.28	**3.11**	-
91	2,2[1]-Methylene bis [6(1,1-dimethyl-4-ethyl]Phenol	2987	-	1.06	-	-	-	**5.25**	2.23	-
92	Heptatriacotanol	3942	-	1.56	0.78		-	-	-	-
	Total		94.32	90.19	72.03	94.67	97.06	98.11	93.01	99.58
	No. of Compounds		46	40	23	26	10	16	11	9
	Monoterpenes		0.88	-	0.72	0.44	2.91	2.17	-	-
	Sesquiterpenes		9.59	10.2	1.56	0.73	-	-	0.47	0.15
	Diterpenes		10.64	10.64	22.16	-	-	-	-	-
	Triterpenes		2.81	3.62	6.57	0.78	-	1.28	3.11	-
	Apocarotenes		15.75	27.8	1.24	0.12	-	-	-	-
	Non-terpenes		54.65	26.41	61.94	92.6	94.15	94.66	89.43	99.43

Key: CPF- *Carica papaya* Fruit, CPL- *Carica papaya* Leaf, CPP- *Carica papaya* Peel, CPRB- *Carica papaya* Root Bark, CPR- *Carica papaya* Root, CPSB- *Carica papaya* Stem Bark, CPS- *Carica papaya* Stem, CPSE- *Carica papaya* Seed RI-Retention Index

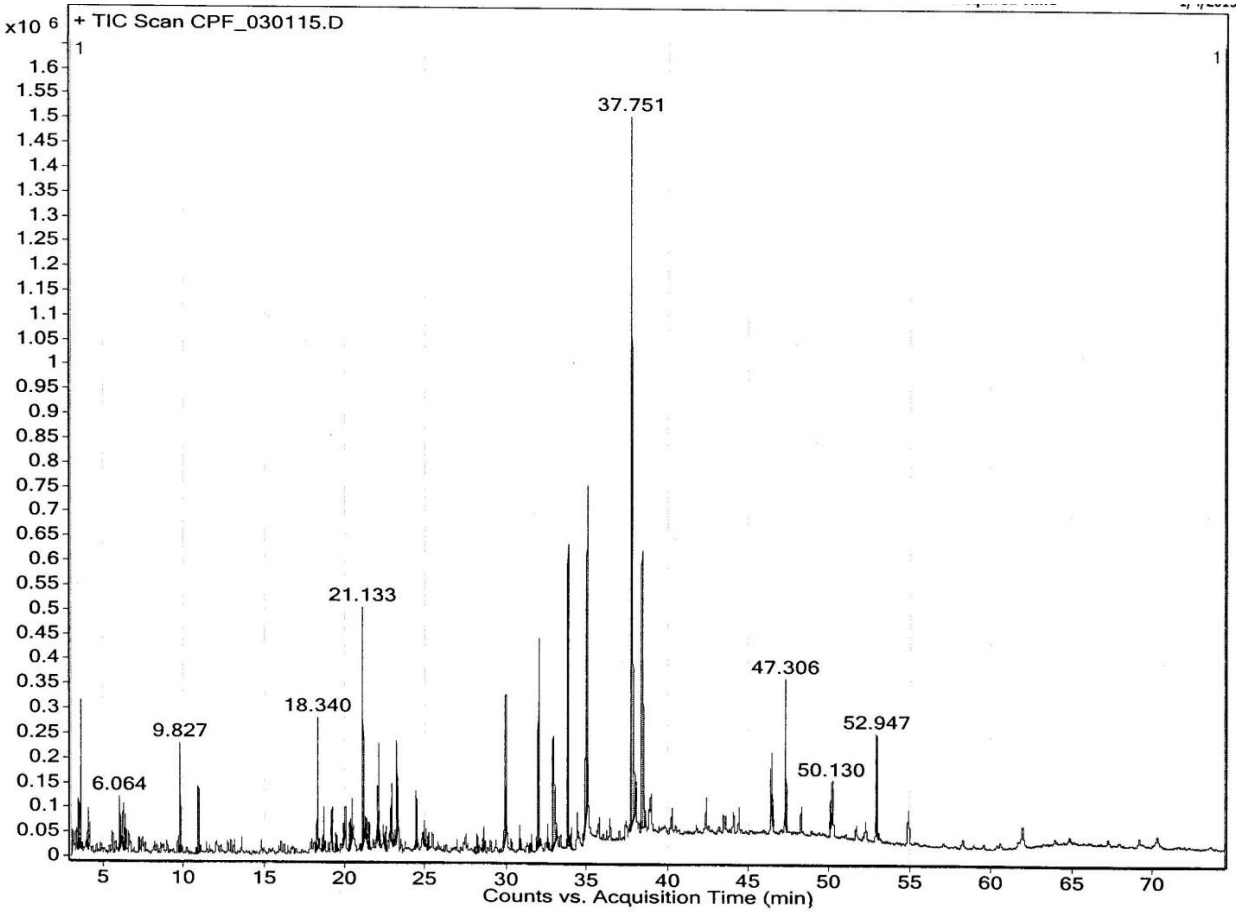


Figure 4.7: GC Chromatogram of *Carica papaya* Fruit

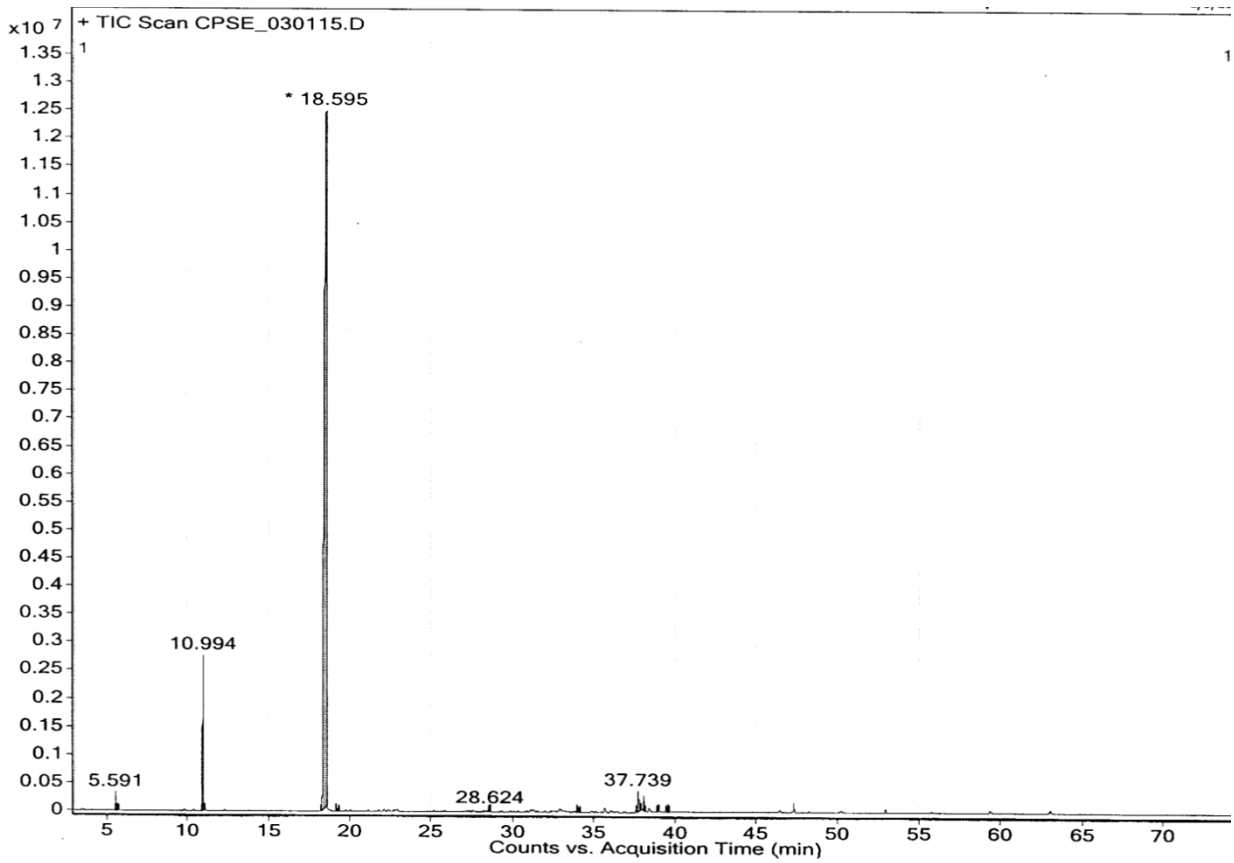


Figure 4.8: GC Chromatogram of *Carica papaya* Seed

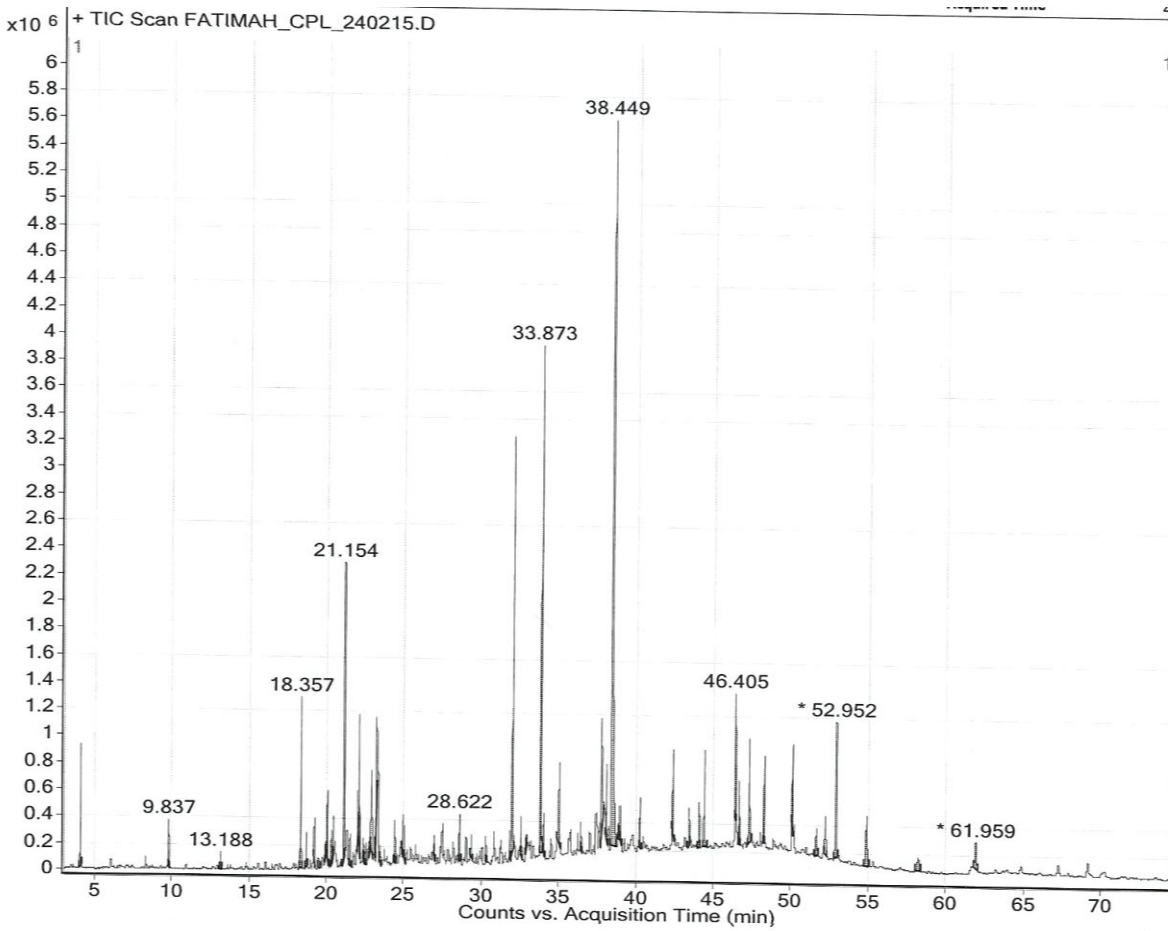


Figure 4.9: GC Chromatogram of *Carica papaya* Leaf

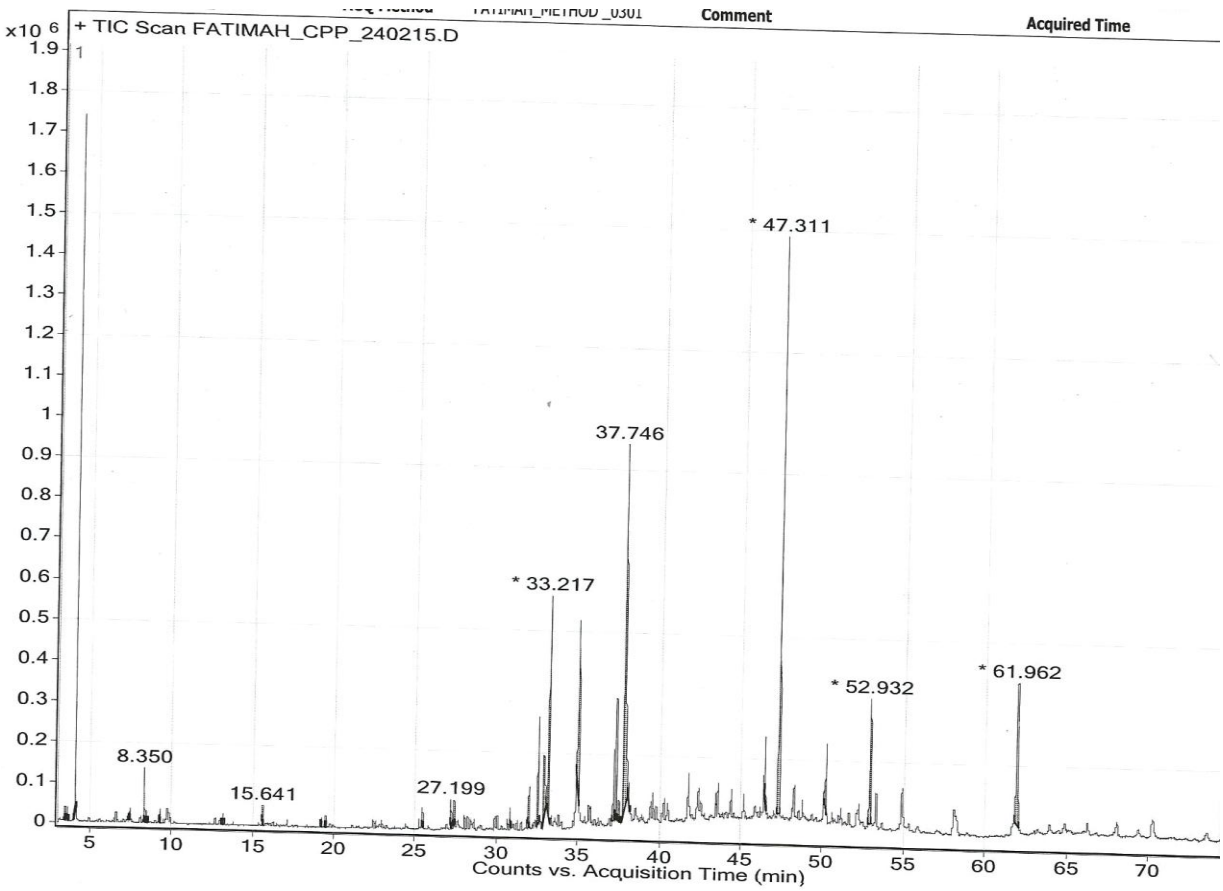


Figure 4.10: GC Chromatogram of *Carica papaya* Peel

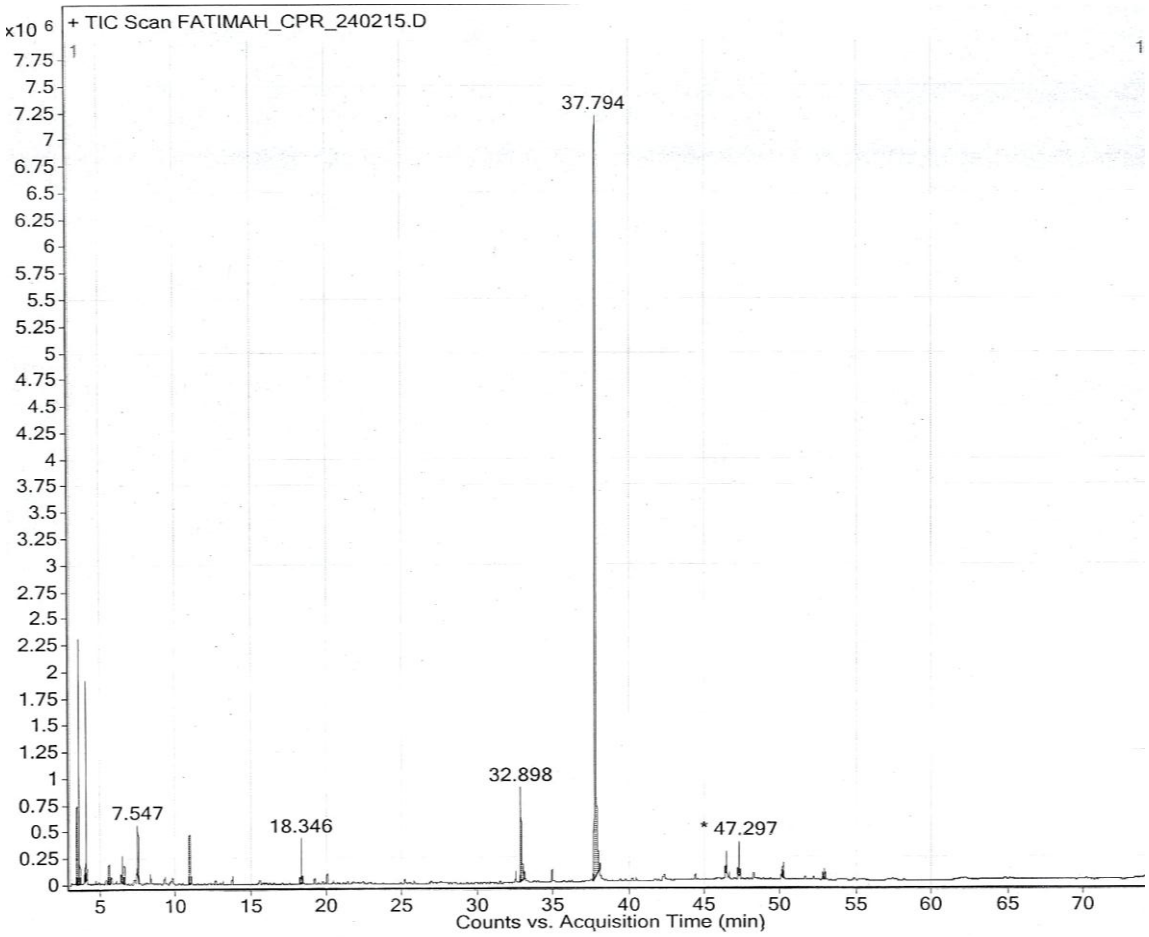


Figure 4.11: GC Chromatogram of *Carica papaya* Root

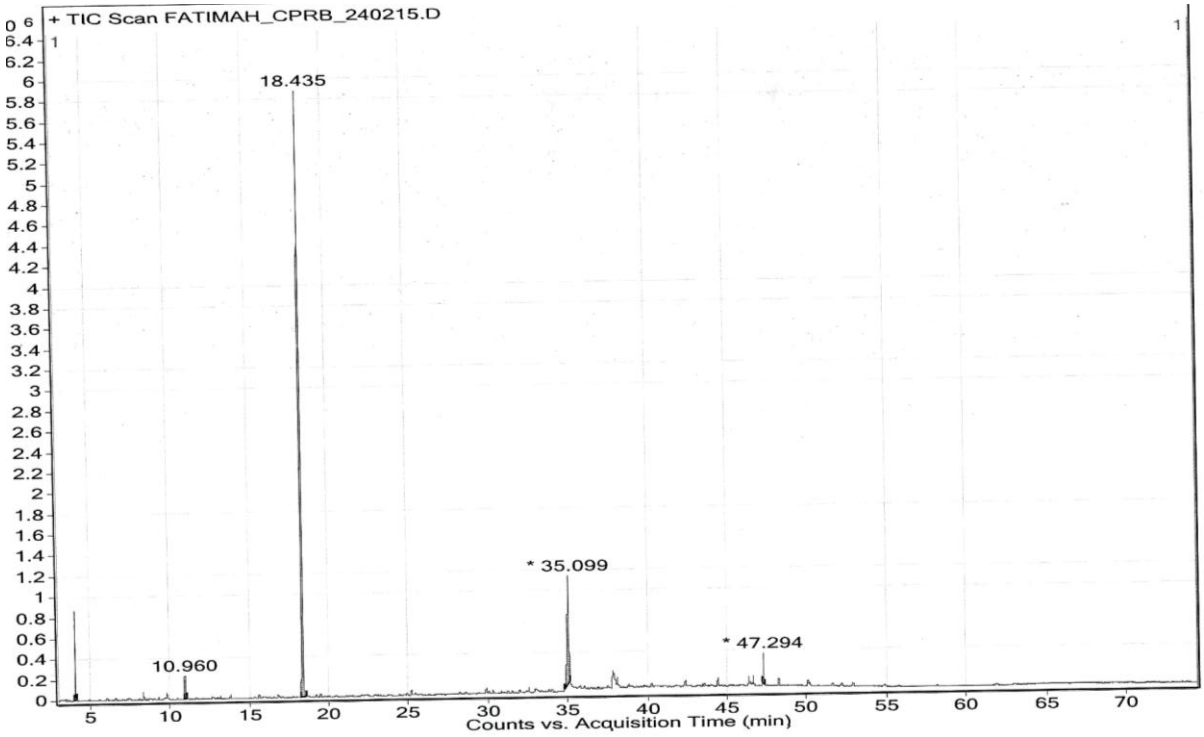


Figure 4.12: GC Chromatogram of *Carica papaya* Root Bark

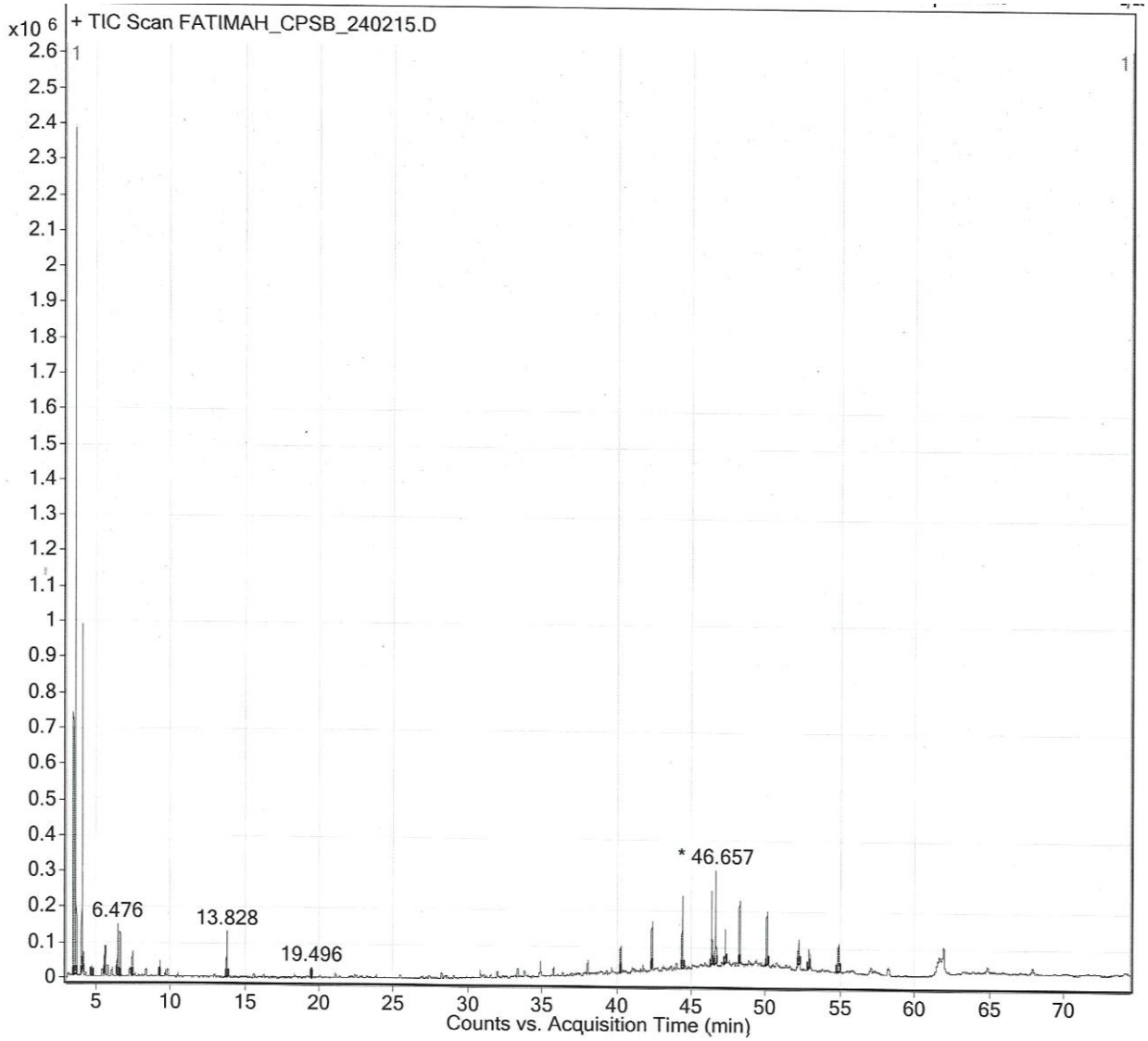


Figure 4.13: GC Chromatogram of *Carica papaya* Stem Bark

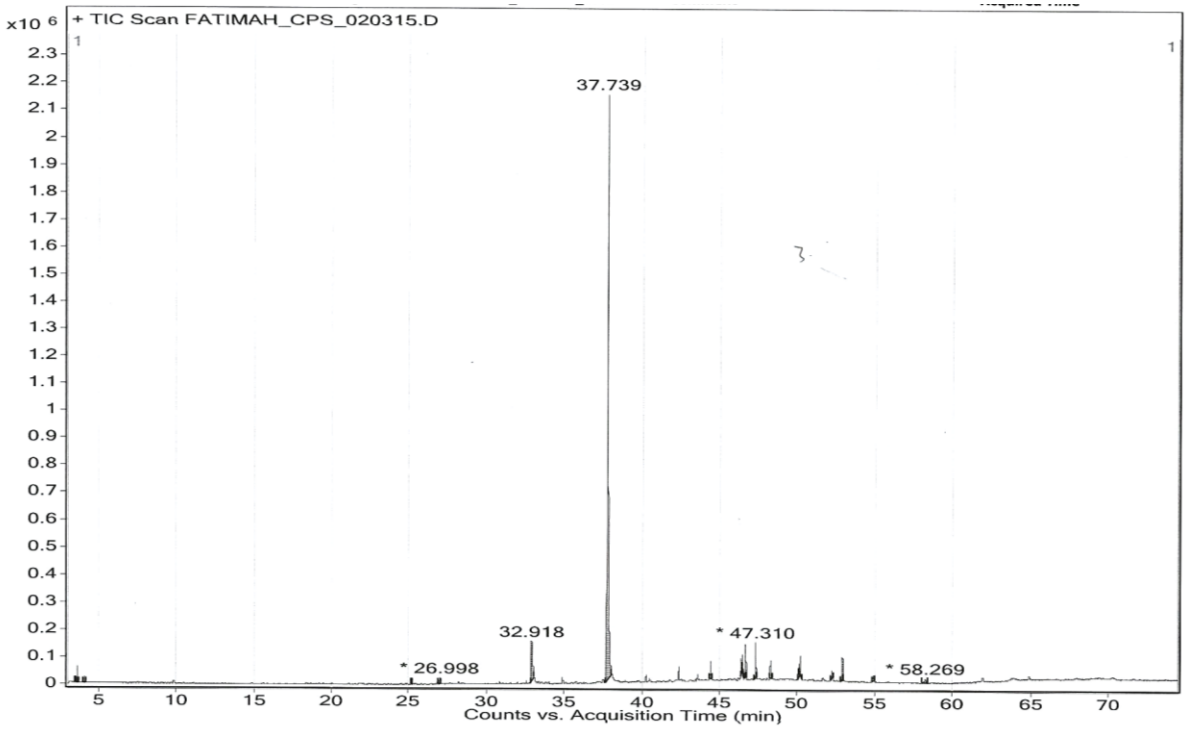


Figure 4.14: GC Chromatogram of *Carica papaya* Stem

4.1.2.3 *Ananas comosus*

The GC (Figures **4.15-4.17**) and GC-MS analysis of essential oils from the fruit, peel and shoot of *Ananas comosus* culminated in the identification of nine, forty-four and thirty-five constituents, which made up 88.76, 66.08 and 91.51 % of the total oil (Table **4.4**). The fruit essential oil comprised predominantly non-terpenes (82.44%). The major components were p-xylene (62.43%), ethylbenzene (12.3%), decane (3.87%) and 1R-α-pinene (3.18%). The peel oil on the other hand had tetradecanoic acid (8.63%), dodecanoic acid (7.77%), γ-palmitoacetone (5.57%), α-copaene (4.2%) and p-xylene (3.09%) as the dominant compounds while p-xylene (29.89%), ethylbenzene (7.64%) and hexadecanoic acid (6.26%) dominated the shoot oil.

The essential oil from the peel had the lowest percentage of monoterpenes (0.84%) and highest percentage of sesquiterpenes (20.87%). Diterpenes are present in only the shoot oil while the peel and shoot oil had triterpene (squalene) present in them at 1.01 and 4.01%. The oils had a high percentage of non-terpenes which were made up of esters, fatty acids, alcohols and aldehydes.

Although ethylhexanoate is an important pineapple fruit aroma compound (Facundo, 2009; Morais and Silva, 2011), it was not present in all the oils. Some other esters were however observed in the peel (ethyldecanoate and ethyl trans-4-decenoate) and the shoot oil (ethylhexadecanoate). The reports by Umano *et al.* (1992); Taivini *et al.* (2001); Elss *et al.* (2005); Akioka *et al.* (2008) and Marta *et al.* (2010) that esters were the major volatile compounds in pineapple volatile composition was not in agreement with the result from this study, however, the report by He *et al.* (2007) that hydrocarbons and esters were the main compounds agrees to an extent with this study. The differences in chemical composition could however be explained by differences in cultivars, growing conditions and volatiles extraction methods (Wei *et al.*, 2011).

The compositional pattern of the essential oils from the shoot and peel of Nigerian grown *A. comosus* are reported here for the first time to the best of my knowledge.

Table 4.4: Essential Oil Components of *Ananas comosus* Fruit, Peel and Shoot

S/N	Compound Name	RI	ACF	ACP	ACSH
1	**Ethylbenzene**	**893**	**12.3**	0.82	**7.64**
2	**p-Xylene**	**907**	**62.43**	**3.09**	**29.89**
3	Nonane	916	1.71	0.34	0.79
4	**1R-α -Pinene**	**948**	**3.18**	-	-
5	S-3-Carene	948	-	0.27	-
6	Cumene	992	-	-	0.6
7	Octanal	1005	-	-	0.4
8	m-Ethyltoluene	1006	-	0.51	2.21
9	**Decane**	**1015**	**3.87**	0.58	1.69
10	D-Limonene	1018	-	0.57	0.5
11	**Hemimelitene**	**1020**	**2.27**	**-**	**3.01**

12	α-Terpinolene	1052	-	-	0.52
13	1-Octanal	1059	-	-	0.41
14	1,8-Cineole	1059	-	-	2.13
15	Trans-2-Nonenal	1112	-	0.4	-
16	1,3,5,8-Undecatetraene	1129	-	0.45	-
17	α-Terpineol	1143	-	-	0.72
18	2-methyldecahydronaphthalene	1162	0.99	-	-
19	Methylnonanoate	1183	-	0.8	2.39
20	Dihydrocarveol	1196	0.87	-	-
21	Decanal	1204	-	0.5	0.73
22	**(-)α-Copaene**	**1221**	**-**	**4.2**	-
23	2-Undecane	1251	-	0.39	-
24	Isoaromadendrene Epoxide	1281	-	0.65	-
25	(+)Sativene	1339	-	1.18	-
26	Ethyldecanoate	1381	-	0.26	-
27	Ethyltrans-4-Decenoate	1389	-	0.62	-
28	p-Eugenol	1392	-	0.24	-
29	(-)β-Elemene	1398	-	0.34	-
30	(-)Aristolene	1403	-	2.1	-
31	Tetradecane	1413	-	0.51	0.38
32	(-)α-Gurjunene	1419	-	0.41	-
33	Geranyl Acetone	1420	-	0.36	0.48
34	γ-Muurolene	1435	-	0.78	-
35	α-Muurolene	1440	-	2.63	-
36	(+)δ-Cadinene	1469	-	1.04	-
37	δ-Guaiene	1490	-	0.8	-
38	α-Himachalene	1494	-	0.99	-
39	β-Caryophyllene	1494	-	-	0.82
40	β-Guaiene	1523	-	1.06	-
41	(+)Ledol	1530	-	-	1.62
42	Epiglobulol	1530	-	2.18	0.71
43	Globulol	1530	-	1.04	-

Table 4.4 continued

S/N	Compound Name	RI	ACF	ACP	ACSH
44	**Dodecanoic Acid**	**1570**	**-**	**7.77**	-
45	Geranylisovalerate	1583	-	0.49	-
46	Caryophyllene oxide	1599	-	0.38	-
47	Tetradecanal	1601	-	-	0.58
48	β-Bisabolol	1619	-	-	0.45
49	Isopropyl-12-methyltridecanoate	1750	-	-	0.73
50	**Tetradecanoic Acid**	**1769**	**-**	**8.63**	1.01
51	Tetradecenoic Acid	1777	-	0.78	-
52	Farnesol acetate	1834	-	0.91	-
53	Hexadecanol	1854	-	-	4
54	Pentadecanoic Acid	1869	-	-	0.58

55	Nonadecane	1910	1.14	-	0.68
56	**Nonanal**	**1910**	-	2.5	**4.73**
57	**Hexadecanoic Acid**	**1968**	-	1.11	**6.26**
58	Ethylhexadecanoate	1978	-	-	2.51
59	**γ-Palmitoacetone**	**1980**	-	**5.57**	-
60	Octadecanol	1999	-	2.66	3.02
61	Trans Phytol	2045	-	-	1.79
62	Heneicosane	2109	-	-	1.63
63	9-Octadecenoic Acid	2175	-	2.2	-
64	9,12-Octadecadienoic Acid	2183	-	1.96	-
65	Heptacosane	2705	-	-	1.89
66	**Squalene**	**2914**	-	1.01	**4.01**
	Total		88.76	66.08	91.51
	No. of Compounds		9	44	35
	Monoterpenes		6.32	0.84	7.48
	Sesquiterpenes		-	20.87	4.08
	Diterpenes		-	-	1.79
	Triterpenes		-	1.01	4.01
	Apocarotenes		-	0.91	-
	Non-terpenes		82.44	42.45	74.15

Key: ACF- *Ananas comosus* Fruit,
ACP- *Ananas comosus* Peel,
ACSH- *Ananas comosus* Shoot RI- Retention Index

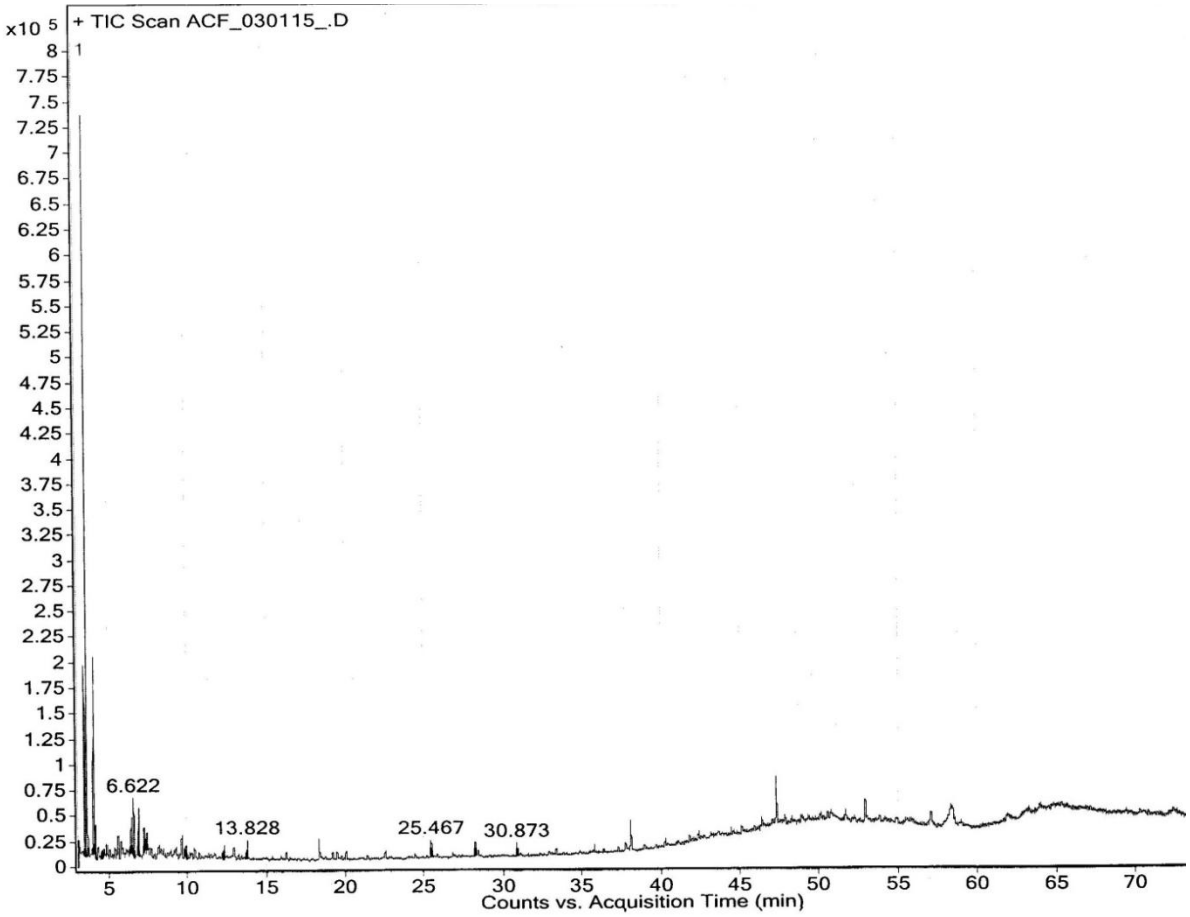


Figure 4.15: GC Chromatogram of *Ananas comosus* Fruit

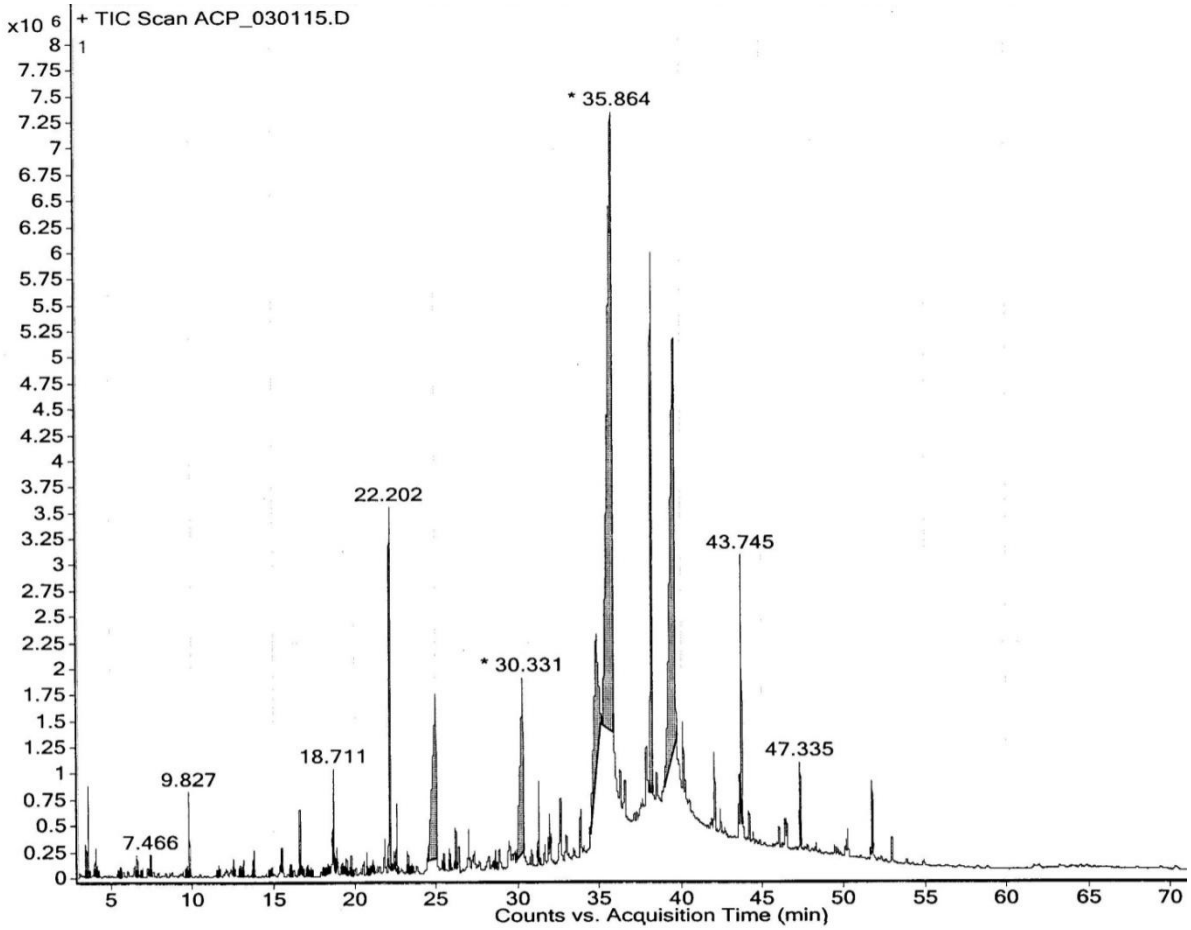


Figure 4.16: GC Chromatogram of *Ananas comosus* Peel

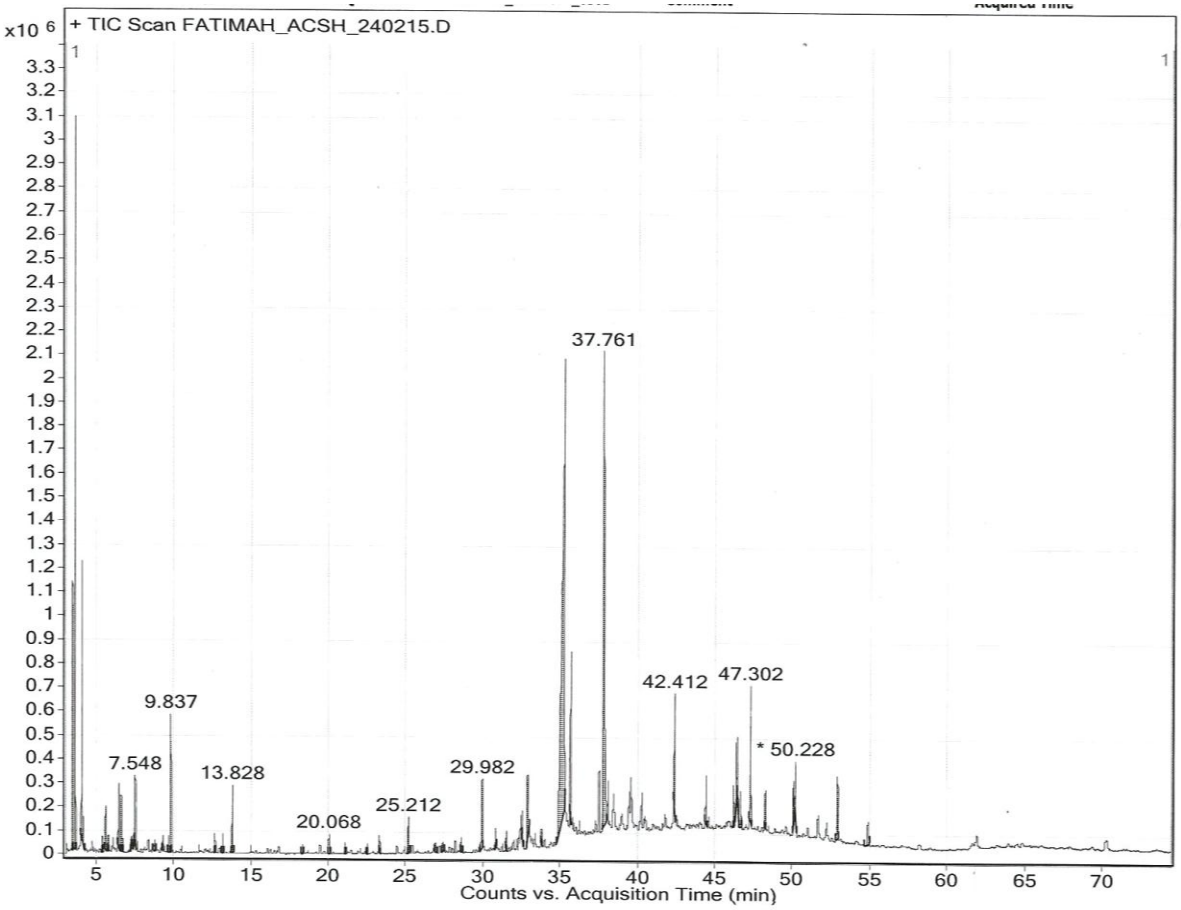


Figure 4.17: GC Chromatogram of *Ananas comosus* Shoot

4.1.2.4 *Theobroma cacao*

The GC (Figure **4.18-4.21**) and GCMS analyses of the colourless essential oil extracted from the hydrodistillation of the leaf, stem bark, pod and seed of *Theobroma cacao* revealed a total of 30, 52, 28 and 19 identified constituents representing 99.8%, 99.2%, 88.43% and 94.45% of the total oils respectively (Table **4.5**). The stem bark

essential oil had 30 sesquiterpenes which made up 68% of the total identified constituents, the peel and leaf oil had 10 and 25 sesquiterpenes representing 40.32% and 15.74% respectively. The seed had low percentage of both monoterpene (3.42%) and sesquiterpenes (4.03%). Diterpenes and triterpenes were present in only the peel oil while apocarotenes were observed in the peel and seed oil only.

The major constituents identified in the leaf oil were hexadecanoic acid (78.69%), octadecanoic acid (4.87%) and epiglobulol (3.93%) while β-caryophyllene (1.16%) and β-bisabolol (0.94%) were amongst the minor compounds. The stem bark oil was dominated by β-bisabolol (17.31%), m-xylene (12.45%), β-bisabolene (6.38%), β-caryophyllene (5.16%), β-sesquiphellandrene (4.61%) and α-bisabolol (4.16%) while ledol (33.62%), α-terpineol (10.12%), heptadecanol (8.98%), farnesyl acetone (5.41%), tetradecanal (4.79%) and 1,8-cineole (4.04%) were the dominant compounds in the pod oil. The seed oil however had o-xylene (53.26%), 3,5-dimethyl octane (5.74%) and hexahydrofarnesyl acetone (4.12%) as its major compounds while nonadecane (4.05%), selin-7(11)-en-4α-ol (4.03%) and nonadecanol (3.57%) were also present in significant quantity.

The GC-MS analysis of *T. cacao* pod oil by the process of soxhlet extraction by Adewole *et al.* (2013) revealed fifteen (15) compounds constituting fatty acids and other organic compounds. However, terpenoids dominated the components in this study and hexadecanoic acid was the only fatty acid present. The difference in composition could be as a result of differences in the extraction method and geographical location of the plant. Although more fatty acids were identified in the essential oils from the leaf and stem bark, only the leaf essential oil had fatty acid as the major compound.

Frauendorfer and Schieberle (2006) reported thirty-five (35) odor-active constituents in cocoa powder based on molecular sensory correlations. Some of the compounds include methylpropanal (malty odor), 2- and 3- methylbutanal (malty odor) and phenylacetaldehyde (honey-like). Although these compounds were not present in the essential oils, the pod oil contains other aldehydes and hexadecanal was present in the seed oil.

The comprehensive study of the essential oil components of *Theobroma cacao* L. leaf, stem bark, pod and seed are been reported for the first time.

Table 4.5: Essential Oil Components of *Theobroma cacao* Linn Plant Parts

S/N	Compound Name	RI	TCL	TCSB	TCP	TCSE
1	Toluene	749	0.29	-	-	-
2	**3,5-Dimethyloctane**	**887**	-	-	-	**5.74**
3	**Ethylbenzene**	**893**	-	**3.63**	-	-

4	**o-Xylene**	**907**	**-**	**-**	**-**	**53.26**
5	**m-Xylene**	**907**	**-**	**12.45**	1.66	**-**
6	**p-Xylene**	**907**	**-**	**4.57**	**-**	**-**
7	Nonane	916	-	0.11	-	-
8	m-Pyrrole	920	-	0.41	-	-
9	Cumene	928	-	0.12	-	-
10	1-Methyl-6-(1-methylethylidine)bicycle[3,1,0]Hexane	956	-	0.17	-	-
11	m-Ethyl toluene	1006	-	0.95	-	
12	Decane	1015	-	0.43	-	0.76
13	D-Limonene	1018	-	0.28	-	1.42
14	Hemimeltene	1020	-	0.18	-	-
15	β-Cymene	1042	-	-	-	0.43
16	α-Terpinolene	1052	-	-	-	1.57
17	**1,8-Cineole**	**1059**	0.15	0.35	**4.04**	-
18	Nonanal	1104	-	0.24	0.49	-
19	Undecane	1115	-	-	-	0.69
20	**α-Terpineol**	**1143**	**-**	**-**	**10.12**	**-**
21	2,3,5,8-Tetramethyldecane	1156	-	-	-	0.85
22	Methyl nonanoate	1183	-	0.35	-	-
23	Decanal	1204	-	-	0.46	-
24	(-)α-Copaene	1221	0.31	1.09	-	-
25	2-Dodecene	1222	-	-	-	0.79
26	2-Undecanone	1251	-	-	0.72	-
27	Undecanal	1303	-	-	0.37	-
28	Tridecane	1313	-	-	-	1.89
29	Benzene isothiocyanate	1318	-	0.34	-	-
30	2-Methylundecanal	1338	-	-	1.31	-
31	Aromadendrene	1386	-	-	0.51	-
32	(-)β-Elemene	1398	-	0.25	-	-
33	α-Cedrene	1403	0.05	1.63	-	-
34	(+)α-Longipinene	1403	0.79	-	-	-
35	**α-Patchoulene**	**1403**	0.19	**3.31**	-	-
36	Geranyl acetone	1420	-	-	1.81	-
37	(+)Epi-β-Santalene	1425	-	0.38	-	-
38	**α-Bergamotene**	**1430**	0.23	**4.19**	-	-

Table 4.5 continued

S/N	Compound Name	RI	TCL	TCSB	TCP	TCSE
39	β-Farnesene	1440	0.14	0.34	-	-
40	α-Amorphene	1440	-	0.21	-	-
41	**β-Sesquiphellandrene**	**1446**	0.25	**4.61**	-	-
42	2-Tridecanone	1449	-	-	0.42	-

43	α-Zingiberene	1451	-	0.18	-	-
44	β-Ionone	1457	-	-	0.36	-
45	Acoradiene	1474	0.51	1.66	-	-
46	Eremophilene	1474	0.15	-	-	-
47	α-Selinene	1474	-	0.31	-	-
48	**β-Caryophyllene**	**1494**	1.16	**5.16**	1.02	-
49	**(+)β-Bisabolene**	**1500**	0.08	**6.38**	-	-
50	(-)β-Caryophyllene oxide	1507	0.18	1.27	2.19	-
51	α-Bisabolene	1518	2.41	1.63	-	-
52	α-Curcumene	1524	2.14	1.55	-	-
53	β-Himachalene	1528	0.11	2.81	-	-
54	**(+)Ledol**	**1530**	0.08	**-**	**33.62**	-
55	**Epiglobulol**	**1530**	**3.93**	-	0.81	-
56	Trans-α-Bisabolene epoxide	1531	-	0.67	-	-
57	α-Cedrol	1543	0.43	1.22	-	-
58	(±)Trans Nerolidol	1564	0.07	1.77	-	-
59	α-Caryophyllene	1579	0.36	0.83	-	-
60	Cubenol	1580	0.42	1.82	-	-
61	Tau-Cadinol	1580	0.12	0.72	-	-
62	(-)δ-Cadinol	1580	0.45	1.04	-	-
63	α-Cadinol	1580	-	1.31	-	-
64	β-Selinenol	1593	-	-	0.36	-
65	**Tetradecanal**	**1601**	**-**	**-**	**4.79**	**-**
66	Hexadecane	1612	-	-	-	2.36
67	**β-Bisabolol**	**1619**	0.94	**17.31**	-	-
68	**α-Bisabolol**	**1625**	0.24	**4.16**	-	-
69	γ-Eudesmol	1626	-	0.19	-	-
70	**Juniper Camphor**	**1647**	-	-	-	**4.03**
71	**Hexahydrofarnesyl acetone**	**1754**	-	-	1.43	**4.12**
72	Tetradecanoic Acid	1769	0.06	0.25	-	-
73	Hexadecanal	1800	-	-	-	1.32
74	Isopropyltetradecanoate	1814	-	-	0.45	-
75	Hexadecanol	1854	-	-	0.72	-
76	**Farnesyl acetone**	**1902**	**-**	**-**	**5.41**	2.51
77	Biformene	1909	-	-	0.52	-

Table 4.5 continued

S/N	Compound Name	RI	TCL	TCSB	TCP	TCSE
78	**Nonadecane**	**1910**	**-**	**-**	**-**	**4.05**
79	**Heptadecanol**	**1954**	**-**	**-**	**8.98**	**-**
80	**Hexadecanoic Acid**	**1968**	**78.69**	**3.54**	1.87	-
81	Methyl-9-Octadecenoate	2085	-	0.26	-	-

82	**Nonadecanol**	**2153**	**-**	**-**	**-**	**3.57**
83	17-Octadecynoic Acid	2165	-	1.03	-	-
84	**Octadecanoic Acid**	**2167**	**4.87**	-	-	-
85	Decyldecanoate	2177	-	-	-	2.13
86	Geranyl Geraniol	2192	-	-	1.03	-
87	Heptacosane	2705	-	1.09	0.89	-
88	Squalene	2914	-	-	2.07	-
89	2,2[1]-Methylenebis(6-tertbutyl-4-ethyl)Phenol	2987	-	0.46	-	2.96
	Total		99.8	99.21	88.43	94.45
	No. of Compounds		30	52	28	19
	Monoterpenes		0.15	0.93	14.16	3.42
	Sesquiterpenes		15.74	68	40.32	4.03
	Diterpenes		-	-	1.55	-
	Triterpenes		-	-	2.07	-
	Apocarotenes		-	-	7.2	6.63
	Non-terpenes		83.91	30.28	23.13	80.37

Key: TCL- *Theobroma cacao* Leaf, TCSB- *Theobroma cacao* Stem Bark,
TCP- *Theobroma cacao* Pod, TCSE- *Theobroma cacao* Seed
RI- Retention Index

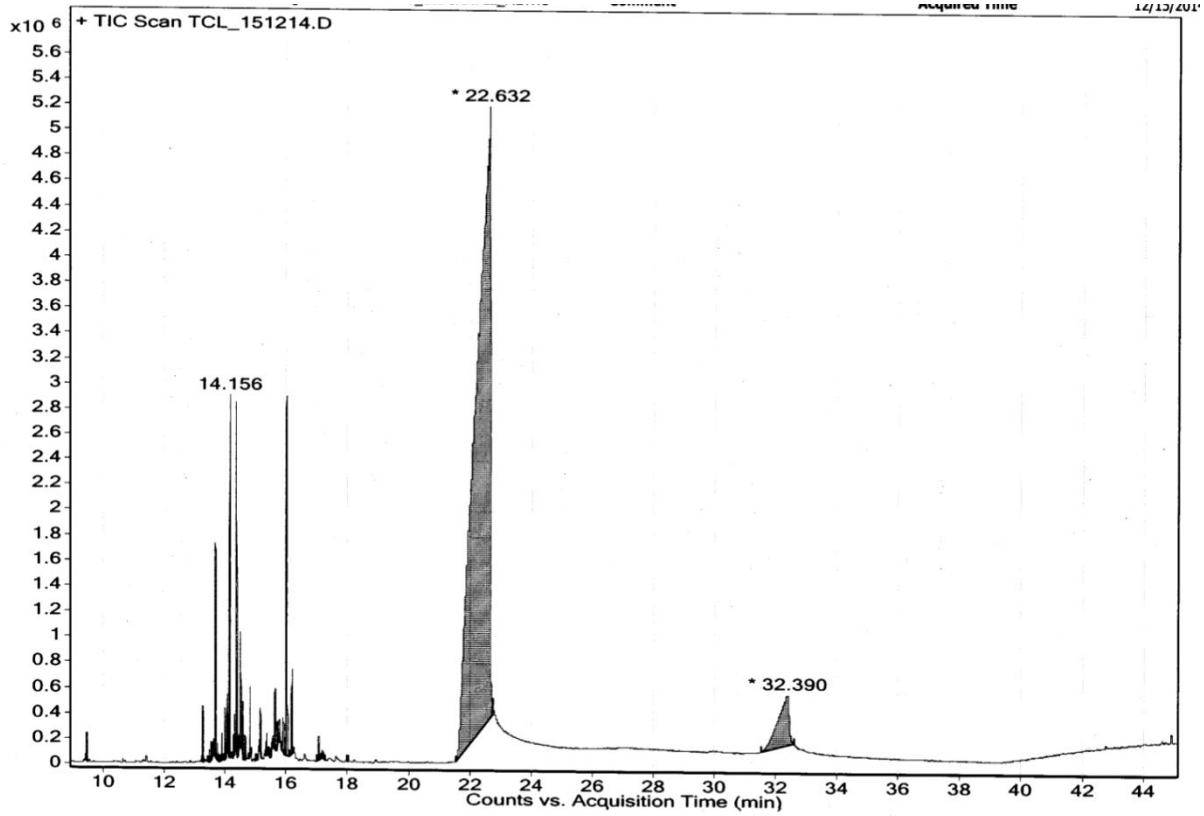


Figure 4.18: GC Chromatogram of *Theobroma cacao* Leaf

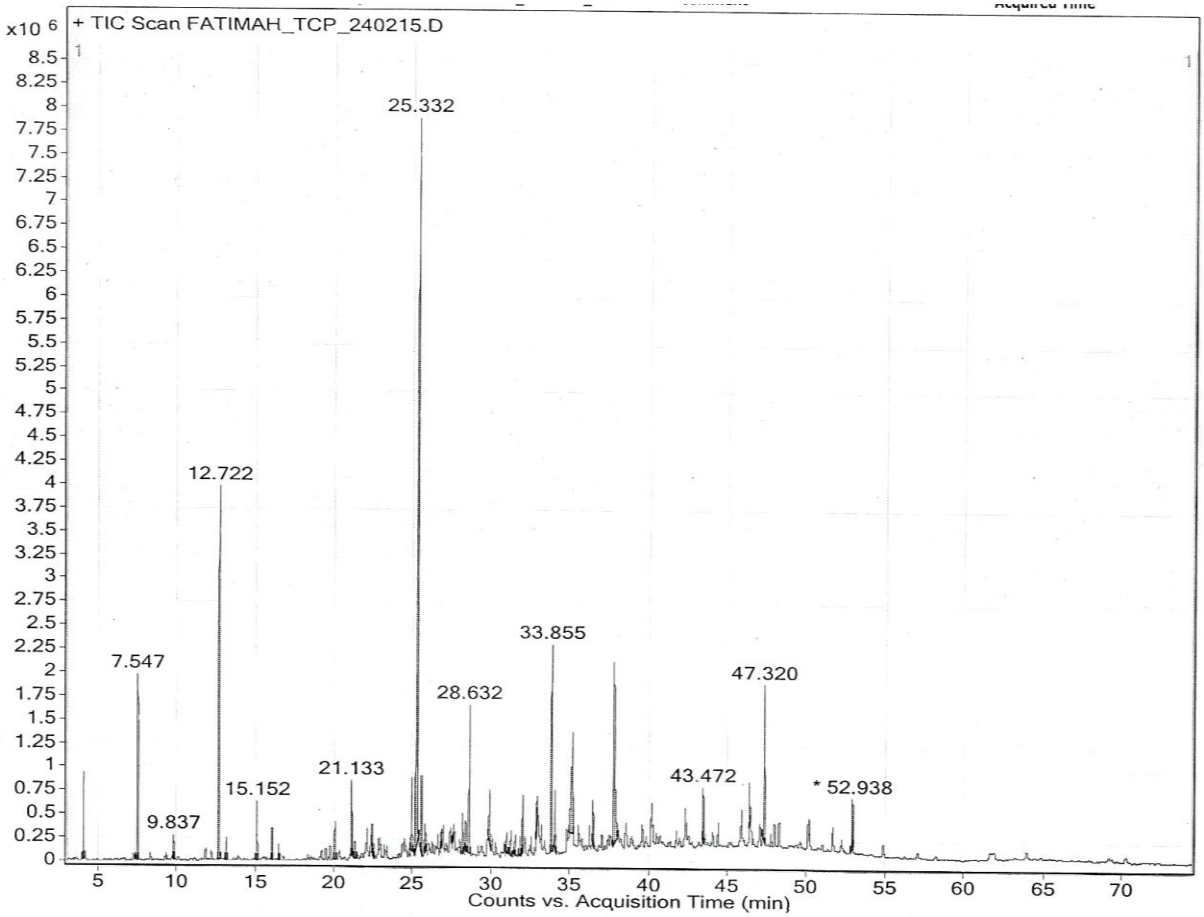


Figure 4.19: GC Chromatogram of *Theobroma cacao* Pod

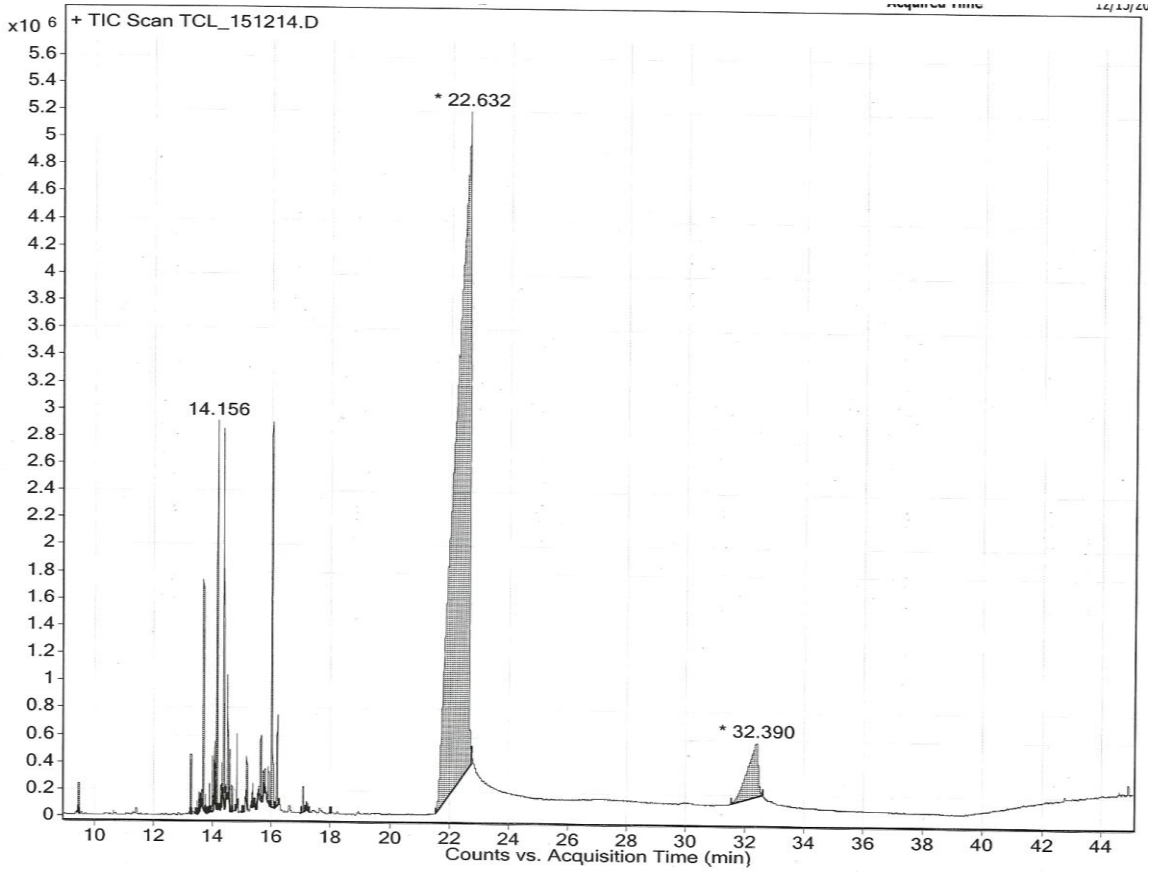


Figure 4.20: GC Chromatogram of *Theobroma cacao* Leaf

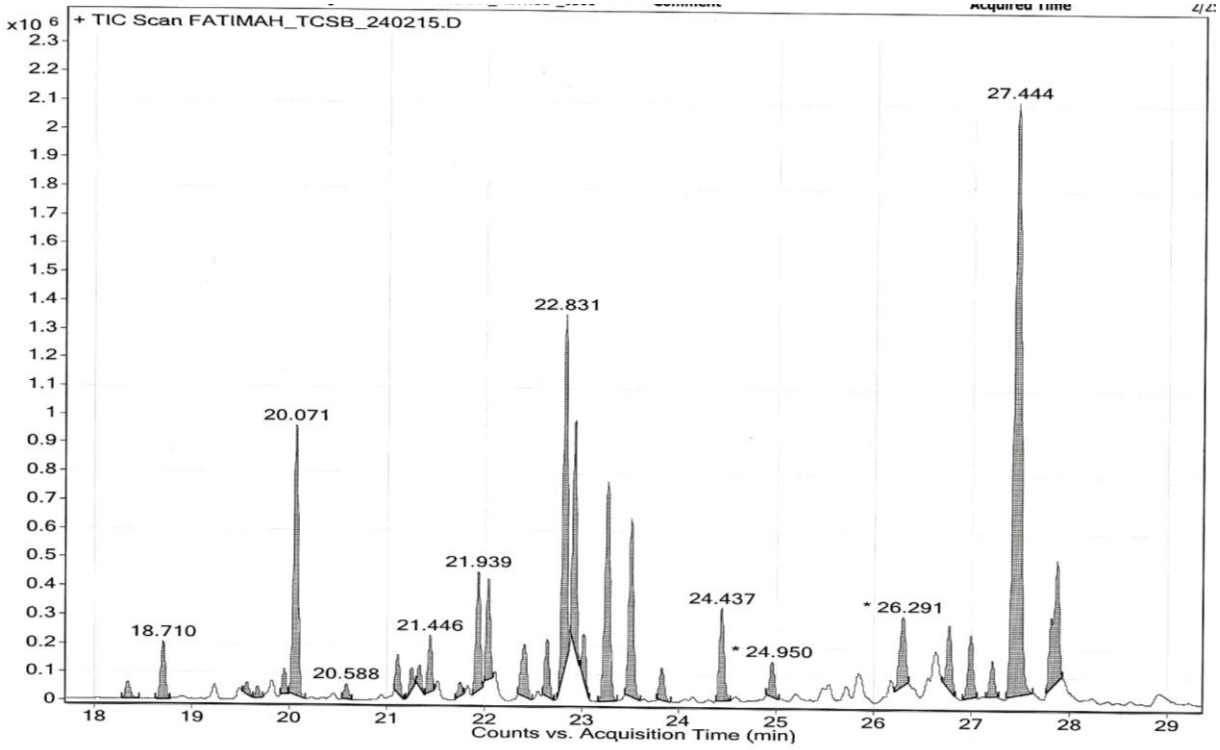


Figure 4.21: GC Chromatogram of *Theobroma cacao* Stem Bark

4.1.2.5 *Chrysophyllum albidium* G. Don

The analyses of the essential oils from the fruit bark, root bark, stem bark, seed bark, leaf and seed of *Chrysophyllum albidium* by GC (Figure **4.23-4.27**) and GCMS showed the presence of 65, 33, 45, 21, 25 and 18 compounds constituting 79.49%, 100%, 90.81%, 98.43%, 96.62% and 98.37% of each of the total oils, respectively (Table **4.6**). Monoterpenes and sesquiterpenes were present in all the oils. The root bark oil had the highest percentage of monoterpene (8.5%) while the fruit bark oil had the least (1.19%). The leaf oil had the highest percentage of sesquiterpenes (75.67%) and the seed bark oil had the lowest quantity (1.3%). Triterpenes were observed in only the fruit bark and stem bark oils while Apocarotenes were detected in all the oils

except the seed oil. The root bark, seed bark and seed oils had 66.9%, 94.24% and 91.15% non-terpene compounds, respectively. All other samples had less than 35% of their components as non-terpenes.

The major compounds in the fruit bark oil were hexadecanoic acid (12.73%), selin-7(11)-en-4α-ol (11.42%), β-elemene (6.74%) and β-bisabolol (3.44%) while the root bark oil was dominated by m-xylene (53.11%), ethylbenzene (9.41%), octadecanol (5.91%) and β-elemene (4.74%). Hexadecanoic acid (14.69%), β-elemene (12.71%), selin-7(11)-en-4α-ol (6.32%) and β-bisabolol (5.52%) were the dominant compounds in the stem bark oil while the seed bark oil was dominated by p-xylene (21.38%), ethylhexadecanoate (19.94%), ethyl-9,12-octadecadienoate (16.91%) and heptacosane (10.51%). α-farnesene (38.11%), β-elemene (7.81%), p-xylene (5.15%) and α-selinene (5.11%) were the major constituents in the leaf oil but m-xylene (66.72%) and undecane (7.16%) dominated the seed oil.

Moronkola *et al.* (2006) reported twenty-four (24) compounds from the GC and GC/MS analysis of the root essential oil of *C. albidum* G. Don with monoterpenes (40.5%) and sesquiterpenes (27.9%) as the dominant class of compounds with pinene (34%), caryophyllene (12.8%), isocaryophyllene (8.5%) and 1,8-cineole (6.5%) as the major compounds. However, the report did not agree with the result of this study. This could be attributed to differences in geographical locations of the plant, sensitivity of instrument used for analysis and moisture content of plant as at time of sampling. Non-terpenes (66.9%) and sesquiterpenes (24.15%) were found to be dominant with m-xylene and β-elemene as the major non-terpene and sesquiterpene compounds, respectively. β-pinene was present in the oil in low quantity. Earlier report on the essential oil of the fruit by Moronkola (2001) presented eight (8) compounds accounting for 90.8% of total components with esters (65.1%) constituting the most abundant class of compounds. A phthalate (dibutyl-1,2-benzenedicarboxylate) was the major compound. The fruit bark analysed in this study however contains more sesquiterpenes (44.56%). The oils were found to contain esters like methylnonanoate, ethylhexadecanoate, ethyl-9-octadecanoate and ethyloctadecenoate which are probably responsible for the sweet, fruity smell of the extracts from the seed bark. The compositional pattern of the essential oils from all the other plant parts of *C. albidum* is here reported for the first time.

Table 4.6: Essential Oil Components of *Chrysophyllum albidum* Plant Parts

S/N	Compound Name	RI	CAFB	CARB	CASB	CASeB	CAL	CASE
1	Methylcyclohexane	781	0.14	-	1.3	-	-	-
2	Toluene	794	1.22	-	3.08	-	-	-
3	3,5-Dimethyloctane	887	-	1.36	-	-	-	1.52
4	**Ethylbenzene**	893	0.09	**9.41**	0.23	**4.4**	-	-
5	**m-Xylene**	907	0.37	**53.11**	0.81	-	-	**66.72**
6	o-Xylene	907	-	-	0.25	-	-	-
7	**p-Xylene**	907	0.16	-	-	**21.38**	**5.15**	**3.02**
8	Nonane	916	0.08	-	-	-	-	-
9	Cumene	928	-	0.83	-	-	-	-
10	(-)β-Pinene	943	0.07	0.52	-	-	-	-
11	1R-α –Pinene	948	0.06	-	-	0.34	-	-

12	S-3-Carene	948	-	0.31	-	-	-	-
13	2,3,6,7-Tetramethyloctane	958	-	-	-	-	-	0.82
14	4,5-Dimethylnonane	986	-	-	-	-	-	1.27
15	α-Decene	1005	-	-	-	-	-	1.09
16	m-EthylToluene	1006	0.09	0.45	0.13	-	-	-
17	Decane	1015	0.08	1.24	-	-	-	1.05
18	D-Limonene	1018	0.03	0.95	-	0.42	-	1.55
19	Trimethylbenzene	1020	0.09	-	-	-	-	-
20	**Hemimelitene**	**1020**	**-**	**3.44**	-	-	-	-
21	β-Cymene	1042	-	0.56	-	-	-	-
22	**α-Terpinolene**	**1052**	-	0.63	-	-	-	**1.77**
23	1,8-Cineole	1059	-	0.78	-	-	-	-
24	β-Linalool	1082	0.56	0.48	0.77	-	1.33	-
25	Nonanal	1104	0.15	0.47	0.45	0.61	1.65	-
26	**Undecane**	**1115**	-	-	-	-	0.45	**7.16**
27	Cyclosativene	1125	-	-	0.14	-	-	-
28	(-)α-Terpineol	1143	0.11	-	-	-	-	-
29	2,3,5,8-Tetramethyldecane	1156	-	-	-	0.42	-	-
30	Indole	1174	0.18	-	0.74	-	-	-
31	Cis-Carvotanacetol	1175	-	-	-	1.21	-	-
32	Methylnonanoate	1183	-	0.45	-	-	-	-
33	Decanal	1204	0.09	-	0.27	-	-	-
34	Dodecane	1215	-	-	-	-	-	0.73
35	(-)α-Copaene	1221	0.13	-	0.67	-	0.57	-
36	Cis-Geraniol	1228	0.06	-	-	-	-	-
37	Isoaromadendrene Epoxide	1281	0.17	-	-	-	-	-
38	4,6-Dimethyldodecane	1285	-	0.41	-	-	-	2.87
39	Calarene Epoxide	1293	0.04	-	-	-	-	-

Table 4.6 continued

S/N	Compound Name	RI	CAFB	CARB	CASB	CASeB	CAL	CASE
40	**Undecanal**	**1303**	-	-	-	-	**3.63**	-
41	α-Tridecene	1304	-	-	-	-	-	1.79
42	2,6,11-Trimethyldodecane	1320	-	-	-	-	-	1.01
43	(-)α-Cubebene	1344	-	0.72	-	-	-	-
44	Aromadendrene	1386	0.49	-	-	-	-	-
45	3-Hexenylhexanoate	1389	-	-	-	-	1.12	-
46	**(-)β-Elemene**	**1398**	**6.74**	**4.74**	**12.71**	-	**7.81**	-
47	α-Cedrene	1403	0.83	1.23	-	-	1.58	-
48	(+)α-Longipinene	1403	0.09	-	0.12	-	-	-
49	α-Patchoulene	1403	0.38	-	0.54	-	-	-

50	α-Gurjunene	1419	0.58	-	1.38	-	1.02	-
51	Geranyl acetone	1420	0.12	-	0.24	-	-	-
52	α-Ionone	1429	-	-	-	-	0.69	-
53	β-Bergamotene	1430	0.06	-	-	-	-	-
54	α-Bergamotene	1430	-	2.01	0.19	-	0.92	-
55	γ-Cadinene	1435	0.05	-	-	-	-	-
56	Cyclohexylhexanoate	1445	-	-	-	-	0.68	-
57	β-Sesquiphellandrene	1446	0.38	-	0.51	-	-	-
58	**α-Farnesene**	**1458**	-	-	-	-	**38.11**	0.88
59	**β-Selinene**	**1469**	**3.32**	1.15	**4.15**	-	-	-
60	(+)δ-Cadinene	1469	-	-	0.32	-	-	-
61	**α-Selinene**	**1474**	2.11	2.13	3.01	-	**5.11**	3.02
62	Acoradiene	1474	-	0.43	0.69	-	-	-
63	**β-Caryophyllene**	**1494**	1.69	2.93	**4.91**	0.37	**8.54**	-
64	α-Himachalene	1494	0.41	-	0.51	-	-	-
65	(±)β-Bisabolene	1500	1.45	2.67	1.42	-	-	-
66	β-Caryophyllene oxide	1507	1.99	-	0.85	-	2.14	-
67	Cis-α-Bisabolene	1518	-	0.37	-	-	-	-
68	2,6,10-Trimethyltetradecane	1519	-	-	-	0.86	-	-
69	**α-Curcumene**	**1524**	**2.56**	0.75	**3.48**	-	-	-
70	β-Himachalene	1528	-	0.71	0.13	-	0.63	-
71	Palustrol	1530	0.29	-	-	-	-	-
72	Epiglobulol	1530	1.24	-	0.87	-	-	-
73	Globulol	1530	0.52	-	0.36	-	1.31	-
74	(+)Ledol	1530	-	-	-	-	1.56	-
75	Veridiflorol	1530	-	-	-	0.45	-	-
76	α-Bisabolene oxide	1531	0.23	-	-	-	-	-
77	Isomethyl-α-ionol	1532	0.12	-	-	-	-	-
78	(-)Calamenene	1537	0.48	-	-	-	-	-

Table 4.6 continued

S/N	Compound Name	RI	CAFB	CARB	CASB	CASeB	CAL	CASE
79	Limonen-6-ol Pivalate	1560	0.11	-	-	-	-	-
80	Hexahydrofarnesol	1563	-	-	0.39	-	-	-
81	Trans Nerolidol	1564	0.21	-	-	-	2.35	-
82	Dodecanoic Acid	1570	1.83	-	1.79	-	-	-
83	γ-Elemene	1570	-	-	-	0.48	-	-
84	**α-Caryophyllene**	**1579**	1.15	0.62	2.13	-	**3.11**	-
85	Cubenol	1580	0.78	-	0.76	-	-	-
86	Tau-Cadinol	1580	0.5	-	0.47	-	-	-
87	Dendrolasin	1607	-	-	-	-	0.91	-
88	**β-Bisabolol**	**1619**	**3.44**	2.79	**5.52**	-	-	-
89	α-Bisabolol	1625	0.54	-	1.32	-	-	-

90	**Juniper Camphor**	**1647**	**11.42**	0.9	**6.32**	-	-	-
91	8-Heptadecene	1719	-	-	-	-	2.71	-
92	**Hexahydrofarnesylacetone**	**1754**	0.52	0.45	0.35	0.92	**3.54**	-
93	**Tetradecanoic Acid**	**1769**	**4.06**	-	2.17	-	-	-
94	**Isopropyltetradecanoate**	**1814**	-	-	-	**3.08**	-	-
95	5-Octadecene	1818	-	-	-	-	-	0.68
96	Hexadecanol	1854	1.23	-	1.91	-	-	-
97	Pentadecanoic Acid	1869	1.81	-	1.27	-	-	-
98	Nonadecane	1910	-	-	-	2.21	-	-
99	Heptadecanol	1954	0.72	-	-	-	-	-
100	**Hexadecanoic Acid**	**1968**	**12.73**	-	**14.09**	-	-	-
101	9-Hexadecenoic Acid	1976	1.6	-	-	-	-	-
102	**Ethylhexadecanoate**	1978	-	-	-	**19.94**	-	1.42
103	**Octadecanol**	**2053**	-	-	**5.91**	-	-	-
104	Heneicosane	2109	-	-	-	3.01	-	-
105	Ethyloctadecanoate	2177	-	-	-	0.51	-	-
106	Cis,cis-9,12-Octadecadienoic Acid	2183	1.12	-	-	-	-	-
107	**Ethyl-9-Octadecenoate**	**2185**	-	-	-	**5.21**	-	-
108	**Ethyl-9,12-Octadecadienoate**	**2193**	-	-	-	**16.91**	-	-
109	**Heptacosane**	**2705**	-	-	-	**10.51**	-	-
110	α-Amyrin	2873	1.05	-	-	-	-	-
111	Squalene	2914	1.44	-	1.18	-	-	-
112	Lupenyl Acetate	2987	2.94	-	-	-	-	-
113	**2,2-Methylene bis [6-(1,1-dimethylethyl)4-ethyl]Phenol**	**2987**	-	-	-	**5.19**	-	-
114	Heptatriacotanol	3942	0.19	-	-	-	-	-
	Total		79.49	100	90.81	98.43	96.62	98.37
	No. of Compounds		65	33	45	19	23	17

Table 4.6 continued

S/N	Compound Name	RI	CAFB	CARB	CASB	CASeB	CAL	CASE
	Monoterpenes		1.19	8.5	1.65	1.97	1.33	3.32
	Sesquiterpenes		44.56	24.15	53.58	1.3	75.67	3.9
	Diterpenes		-	-	-	-	-	-
	Triterpenes		5.43	-	1.18	-	-	-
	Apocarotenes		0.64	0.45	0.74	0.92	4.23	-
	Non-terpenes		27.67	66.9	33.66	94.24	15.39	91.15

Key: CAFB- *Chrysophyllum albidum* Fruit Bark, CARB- *Chrysophyllum albidum* Root Bark, CASB- *Chrysophyllum albidum* Stem Bark, CASeB- *Chrysophyllum albidum* Seed Bark, CAL- *Chrysophyllum albidum* Leaf, CASE- *Chrysophyllum albidum* Seed
RI- Retenton Index

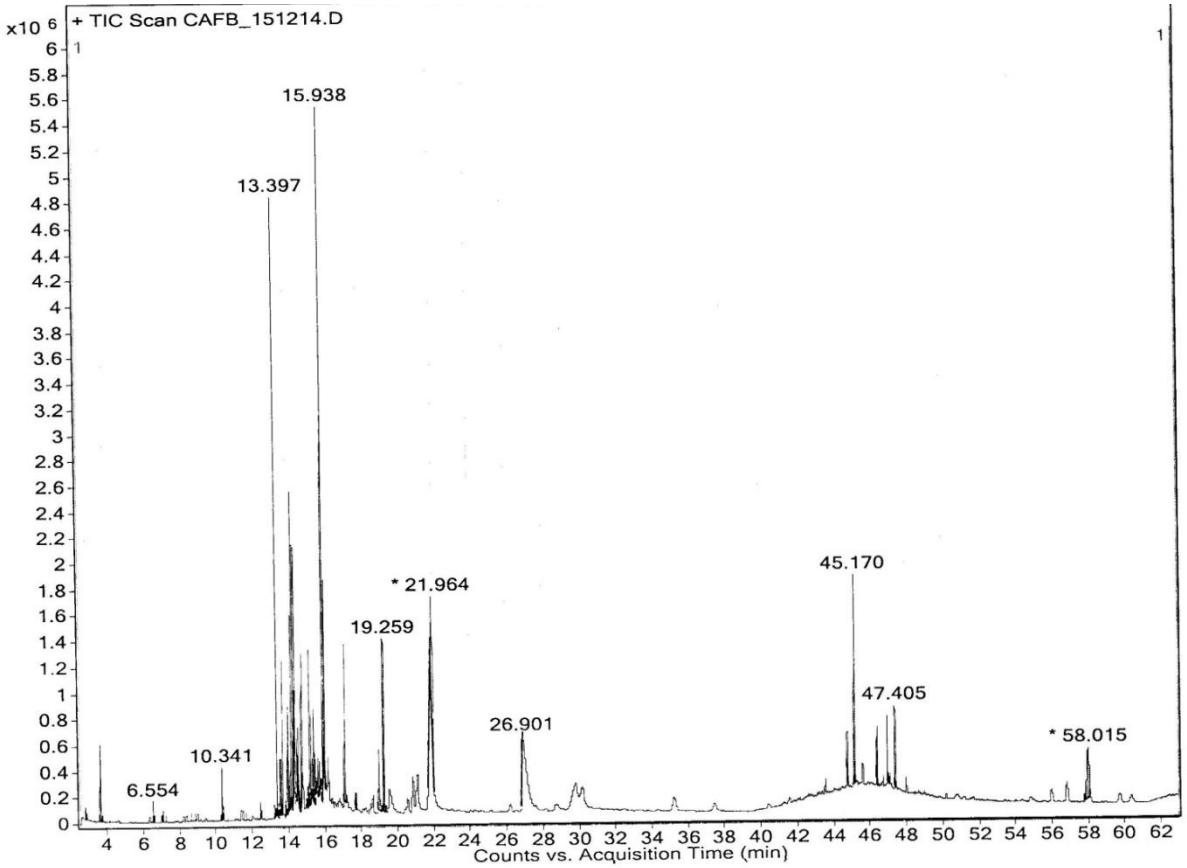


Figure 4.22: GC Chromatogram of *C. albidum* Fruit Bark Oil

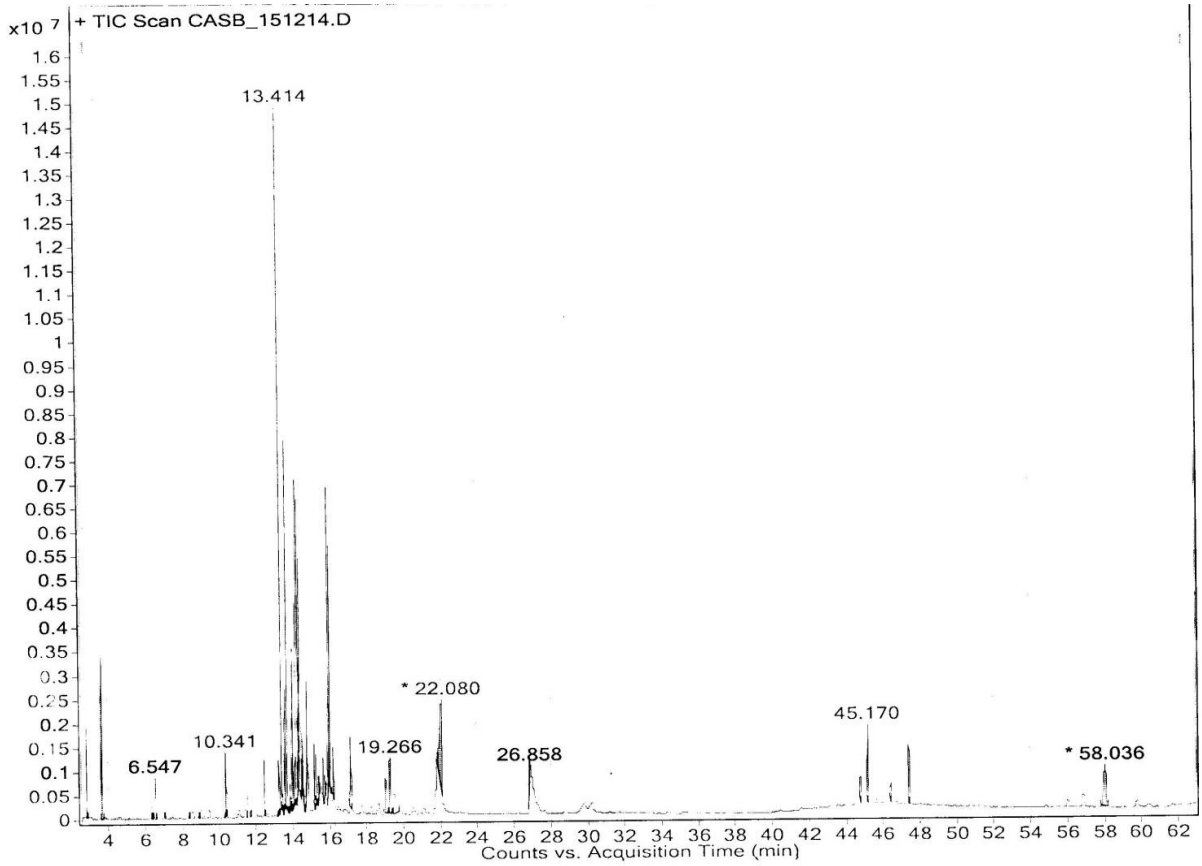


Figure 4.23: GC Chromatogram of *C. albidum* Stem Bark Oil

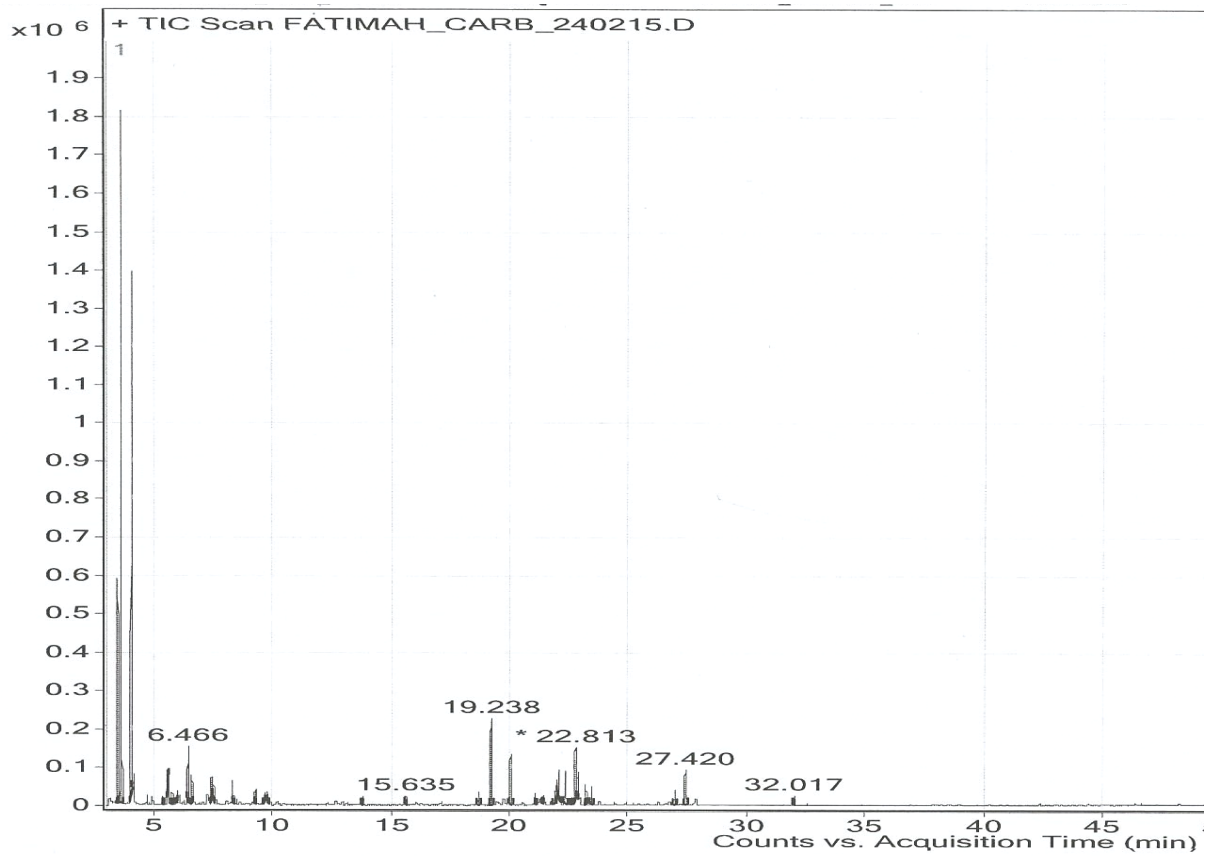


Figure 4.24: GC Chromatogram of *C. abidum* Root Bark Oil

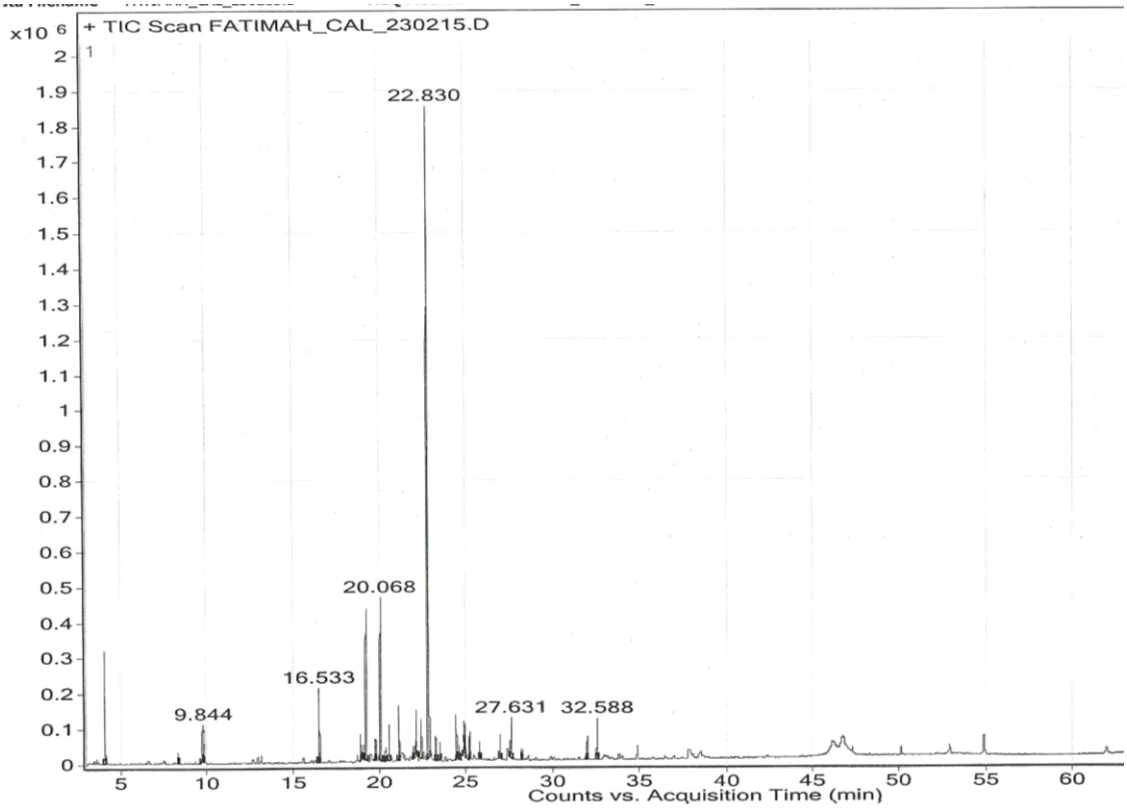


Figure 4.25: GC Chromatogram of *C. abidum* Leaf Oil

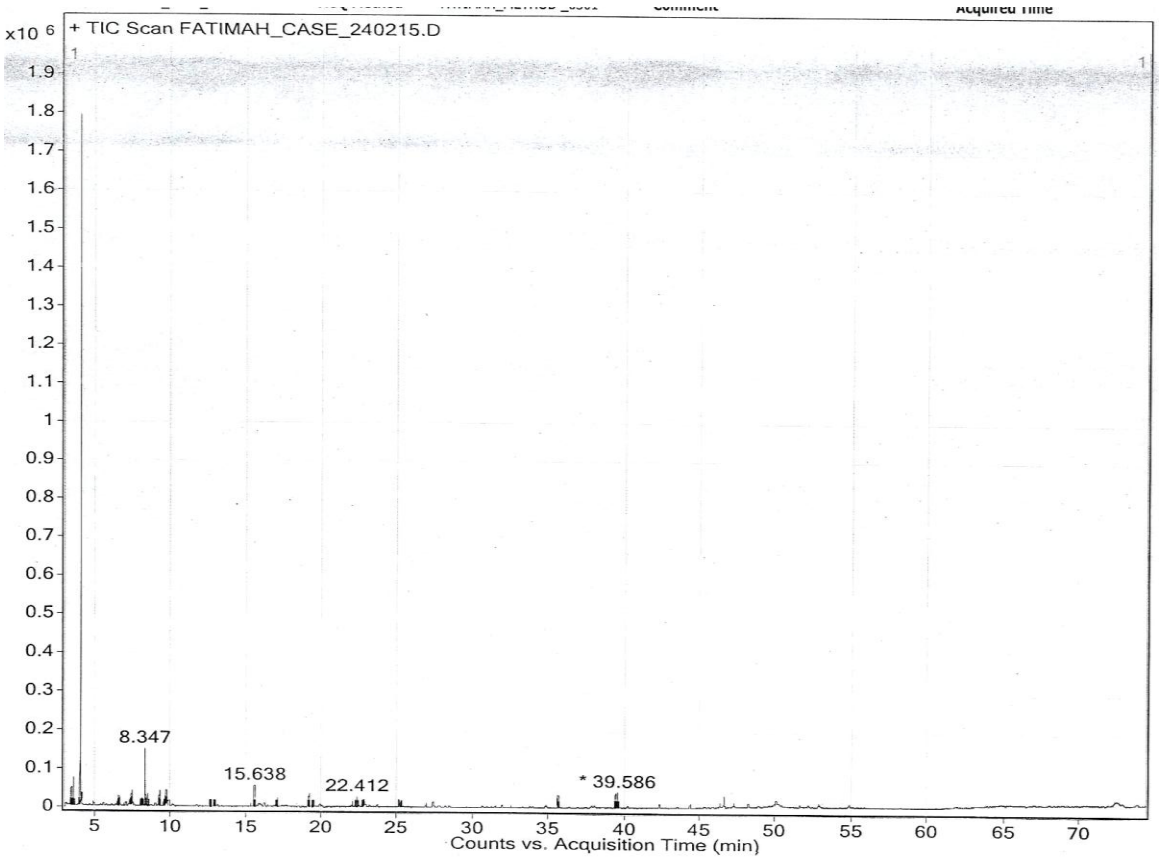


Figure 4.26: GC Chromatogram of *C. abidum* Seed Oil

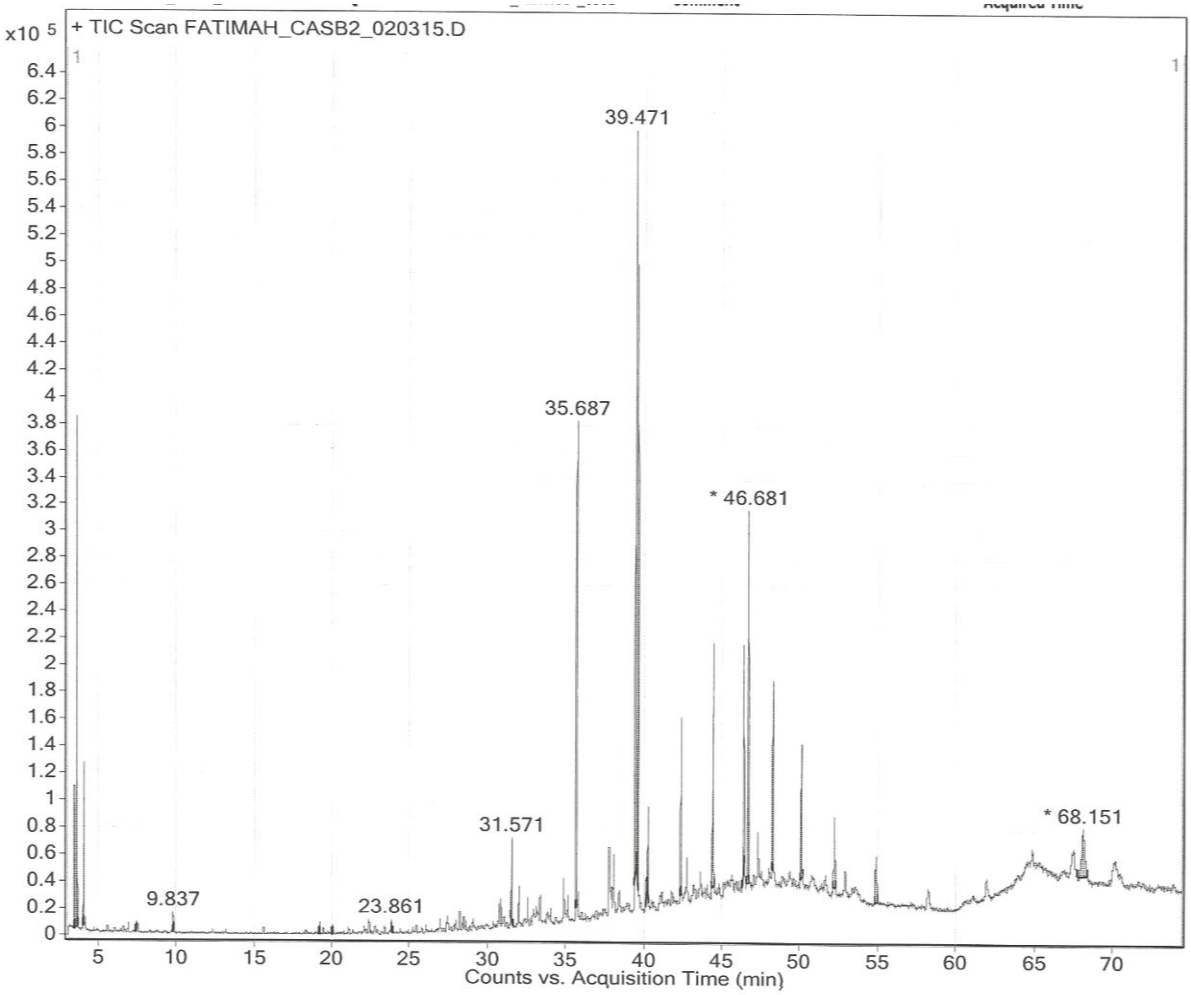


Figure 4.27: GC Chromatogram of *C. abidum* Seed Bark Oil

4.2 Non-Volatile Extracts

4.2.1 Percentage Yield of Non-Volatile Extract

The leaves had higher yields compared to all the other parts as presented in Table **4.7**. The yields range was between 0.08 and 11.62%. The seeds had very low yield except

for *T. cacao* seed. All the leaf extracts had greenish colour while the stem and root barks had brownish colours except for the stem and root bark of *C. papaya*.

Table 4.7: Percentage Yield of Non-Volatile Extracts

S/N	Plant Sample	Part used	Extract Yield (%w/w)
1	*Persea americana*	Stem Bark	7.19

		Root Bark	9.25
		Leaf	11.62
		Peel	5.78
		Seed	3.41
2	*Carica papaya*	Root Bark	2.42
		Stem Bark	2.23
		Seed	0.73
		Leaf	5.61
		Peel	4.01
3	*Ananas comosus*	Shoot	2.85
		Peel	4.02
4	*Theobroma cacao Linn.*	Stem Bark	5.13
		Leaf	5.82
		Pod	1.49
		Seed	6.23
5	*Chrysophyllum albidium*	Stem Bark	2.6
		Root Bark	2.88
		Leaf	11.43
		Seed	2.91
		Seed Bark	0.08
		Fruit Bark	4.12

4.2.2 Phytochemical Screening of Non-Volatile Extracts

All the 22 samples screened for secondary metabolites showed the presence of alkaloids, saponins and flavonoids with varying observation of anthocyanins, steroids,

glycosides and anthraquinones (Table **4.8**). Reducing sugar was not detected in all extracts of the fruit plants indicating that the reducing sugar in fruits are more concentrated in the juicy part of the fruit and not the other parts of the tree.

This result is in agreement with earlier reports on the leaves of *C. albidum* G. Don (Amusa *et al.*, 2003; Okoli and Okere, 2010; Orijajogun *et al.* 2013; Kamba and Hassan, 2011) with respect to the presence of alkaloids, tannin, saponin, phenol and flavonoid. The results obtained showed that *P. americana* Mill contain phytochemicals such as saponins, tannins, steroids, alkaloids and flavonoids corresponding to earlier report by Arukwe *et al.* (2012). *T. cacao* Linn was found to be rich in saponins, alkaloids, flavonoids and tannins. This is in accordance with previous study by Zainal *et al.*, 2014, Ogunmefun *et al.*, 2013, Izuka and Mbangwu, 2013 and Nwokonkwo and Okeke, 2014). Phytochemical analysis of *C. papaya* revealed the detection of flavonoids, alkaloids, steroids and tannins in the methanol extract. This corresponds to earler report by Khaled *et al.* 2013) and Anjum *et al.* (2013). The groups of secondary metabolites in each of the extracts are known to have a variety of biological activities which could be responsible for the ethnomedicinal use of the fruit plants. The preliminary phytochemical analysis (Table **4.8**) of the extracts of *A. comosus* shoot and peel are reported for the first time to the best of my knowledge.

Table 4.8: Phytochemicals of the Non-volatile Extracts of the Fruit Tree Parts

Code	Alk	Antho	Tan	Gly	Sap	Ste	Flav	Anthra	Red. Sug
PASB	+	+	ND	+	+	+	+	+	ND

PARB	+	+	ND	+	+	+	+	+	ND
PAL	+	+	+	ND	+	+	+	ND	ND
PAP	+	+	ND	+	+	+	+	+	ND
PASE	+	ND	ND	+	+	+	+	+	ND
CPRB	+	ND	+	ND	+	ND	+	ND	ND
CPSB	+	ND	+	+	+	+	+	+	ND
CPSE	+	ND	ND	ND	+	+	+	ND	ND
CPL	+	+	+	+	+	+	+	+	ND
CPP	+	+	ND	+	+	+	+	+	ND
ACSH	+	+	+	ND	+	+	+	ND	ND
ACP	+	+	+	ND	+	+	+	ND	ND
TCL	+	ND	+	ND	+	+	+	ND	ND
TCSB	+	+	+	+	+	+	+	+	ND
TCP	+	+	+	+	+	+	+	+	ND
TCSE	+	+	+	+	+	+	+	+	ND
CASB	+	+	+	+	+	+	+	+	ND
CARB	+	+	+	ND	+	+	+	+	ND
CAL	+	ND	+	ND	+	+	+	ND	ND
CASE	+	ND	+	ND	+	+	+	ND	ND
CASeB	+	ND	+	ND	+	ND	+	ND	ND
CAFB	+	+	+	ND	+	+	+	ND	ND

Key: ND-Not Detected, + - Present, Alk- Alkaloids, Antho- Anthocyanins, Tan- Tannins, Gly- Glycosides, Sap- Saponins, Ste- Steroids, Flav- Flavonoids, Anthra- Anthraquinones, Red. Sug- Reducing Sugar
PASB- *P. americana* Stem Bark, PARB- *P. americana* Root Bark, PAL- *P. americana* Leaf, PAP- *P. americana* Peel, PASE- *P. americana* Seed,
CPRB- *C. papaya* Root Bark, CPSB- *C. papaya* Stem Bark, CPSE- *C. papaya* Seed, CPL- *C. papaya* Leaf, CPP- *C. papaya* Leaf,
ACSH- *A. comosus* Shoot, ACP- *A. comosus* Peel,
TCL- *T. cacao* Leaf, TCSB- *T. cacao* Stem Bark, TCP- *T. cacao* Pod, TCSE- *T. cacao* Seed, CASB- *C. albidum* Stem Bark, CARB- *C. albidum* Root Bark, CAL- *C. albidum* Leaf,
CASE- *C. albidum* Seed, CASeB- *C. albidum* Seed Bark, CAFB- *C. albidum* Fruit Bark

4.3 Isolation of Compounds from *T. cacao* L. Pod

4.3.1 Spectroscopic Analysis of Compound 2TCHD-3

Compound 2TCHD-3 (2.6 mg) (Figure **4.28**) appeared as white solid with a melting point of 112-114 ^{o}C. The IR absorption bands (Figure **4.29**) show the presence of ketone ($V_{C=O}$ stretching 1706 cm^{-1}), olefin ($V_{C=C}$ stretching 1654) and aliphatic hydrocarbons (V_{CH} stretching 2941 and 2869 cm^{-1}) as seen on Table **4.9**. The low resolution EI-MS (Figure **4.30**) showed m/z 424.4 (M^{+}-H_2O) corresponding to a molecular formular $C_{30}H_{46}O$. The mass spectrum showed fragment ions at *m/z* 313 [M - $C_8H_{13}O$ (side chain)]$^{+}$ and 175 [M - $C_{17}H_{27}O_2$ (side chain + ring A)]$^{+}$.

The 1H NMR (Figure **4.31**) spectrum showed five methyl singlets (δ_H 0.89, 0.98, 1.03, 1.08, 1.78), a secondary methyl group at δ_H 0.87 (3H, d, *J*= 5.6 Hz), terminal methylene protons at δ_H 4.87 (1H, br, s) and 4.98 (1H, br, s) and two doublet protons for a cyclopropyl CH_2 group at δ_H 0.55 (1H, d, *J*= 4.3 Hz) and 0.78 (1H, d, *J*= 4.3 Hz), indicating a cycloartane skeleton. 1H NMR data reveals the presence of an OH group on C-24 δ_H 3.47 and an oxymethine δ_H 4.34 (1H, t, *J*= 6.6 Hz). This oxymethine proton was assigned at C-24 due to its chemical shift and coupling pattern as well as HMBC correlation to C-25, C-26, and C-27. The signals at δ_C 216.7, δ_C 144.3 and δ_C 114.3 were due to the presence of quaternary carbon with an oxo group at C-3 and olefinic carbons at C-25 and C-26 respectively (Figure **4.32**). The DEPT 90 (Figure **4.33**) DEPT 135 (Figure **4.34**), HSQC (Figure **4.35**), HMBC (Figure **4.36**), and COSY (Figure **4.37**) showed thirty carbon signal including six methyls, twelve methylenes, five methine and seven quaternary carbons. The HMBC spectrum (Figure **4.36**) showed long range correlations from H-22, H-26, and H-27 to C-24; H-19 to C-8, C-9, C-11 and C-12; and between H-30 to C-8, C-13, C-14 and C-15. On the other hand, in the HMBC spectrum, the signals of H-28 and H-29 correlated with that of the oxo group (δC_{-3} 216.7), indicating that the oxo group was located at C-3 and which caused the H-19 signals of the cyclopropane ring to appear downfield to δH 0.76 (d, *J*= 4.3 Hz) and 0.55 (d, *J*= 4.3 Hz), respectively.

Hence, the structure of 2TCHD-3 was assigned as 24-hydroxy-25-cycloarten-3-one with spectroscopic data (Figure **4.28**) that was consistent to reported literature values (Chiu *et al.*, 2008) as shown on Table **4.10**.

Cycloartanes have been reported to have cytotoxic and leishmanicidal activities (Lavoie *et al.*, 2013; Choudhary *et al.*, 2008). Zahid *et al.* (2007) reported that

cycloartanes exhibited acetylcholinesterase inhibition which implies their possible application in the treatment of Alzheimer's disease.

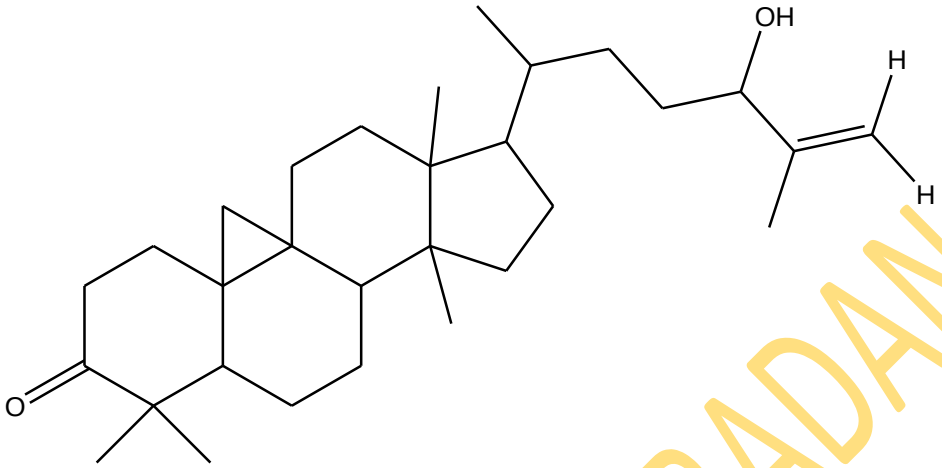


Figure 4.28: Compound 2TCHD-3 (24-hydroxy-25-cycloarten-3-one)

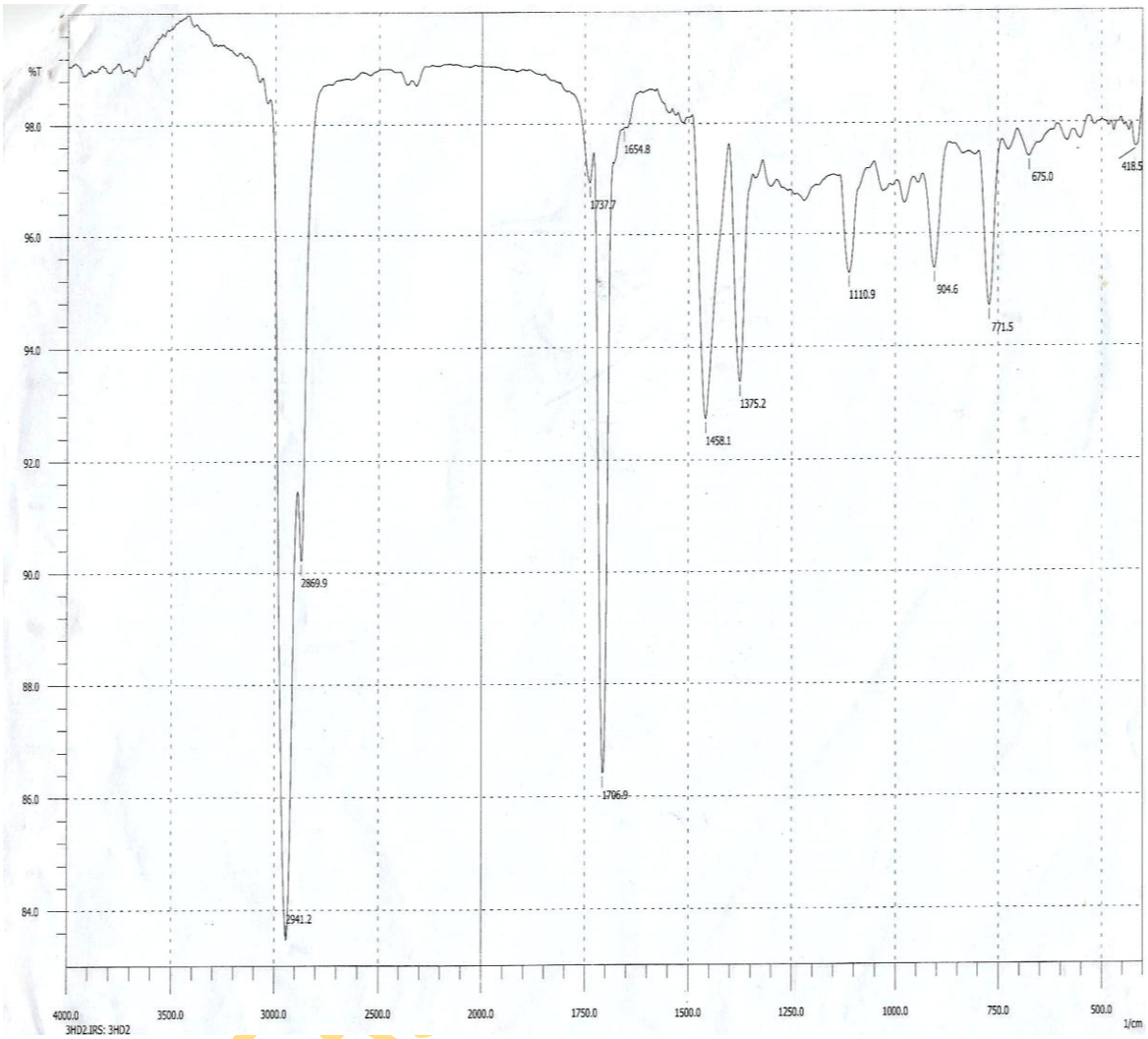


Figure 4.29: IR Spectrum of Compound 2TCHD-3

Table 4.9: Infra Red values of 2TCHD-3

Functional Group	IR values (V/cm^{-1})

C=C (Olefin)	1654
C=O (Ketone)	1706
C-H (Aliphatic Stretch)	2941 and 2869

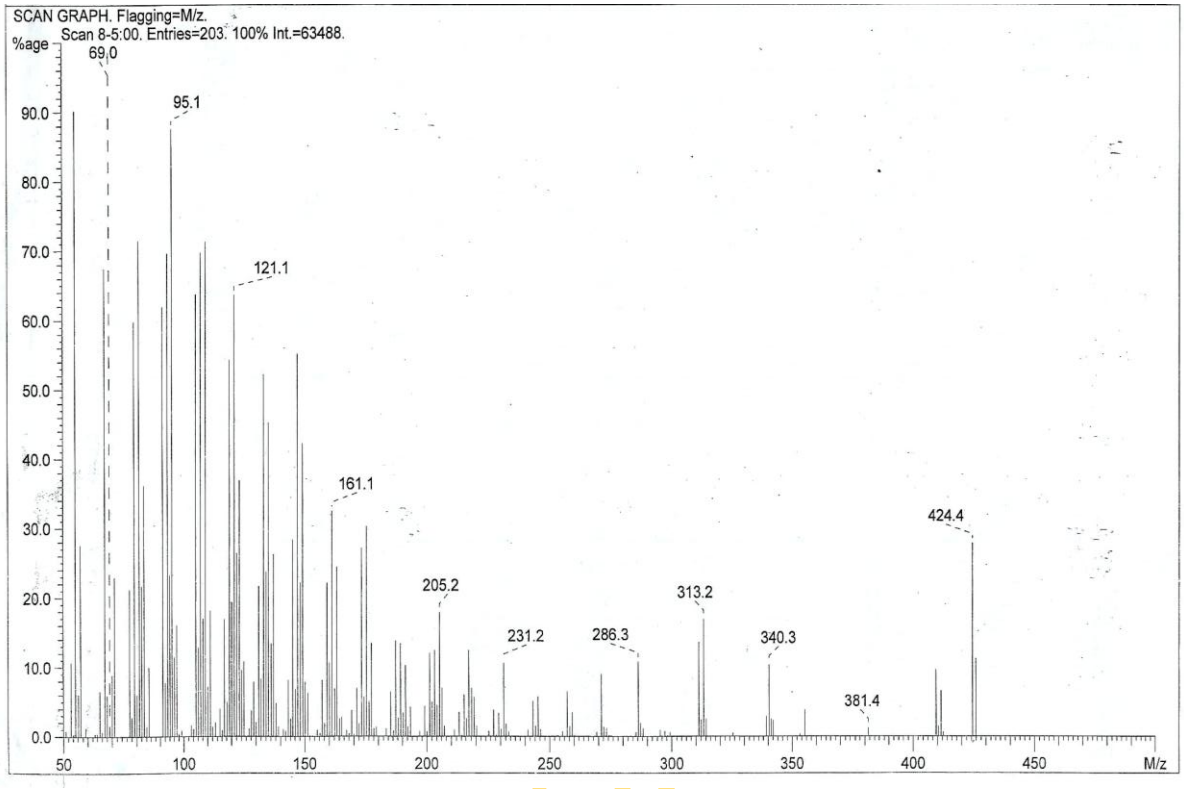


Figure 4.30: EIMS Spectrum of Compound 2TCHD-3

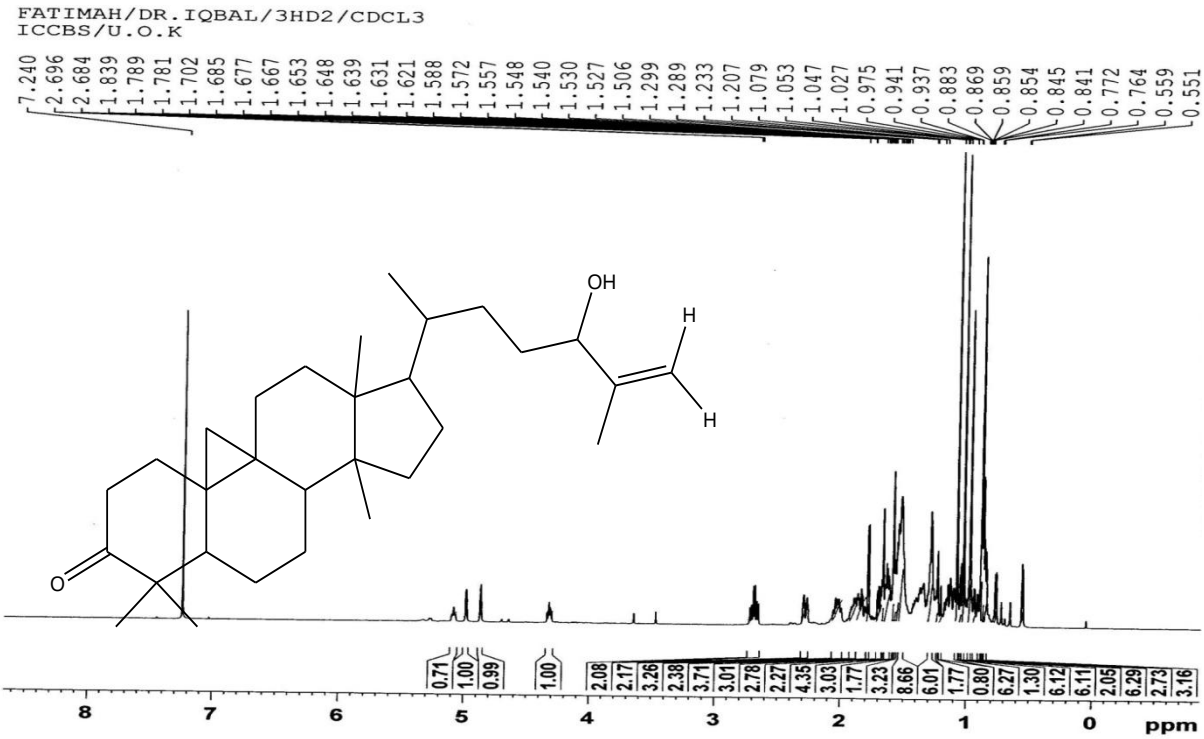


Figure 4.31: ^{1}H NMR Spectrum of Compound 2TCHD-3

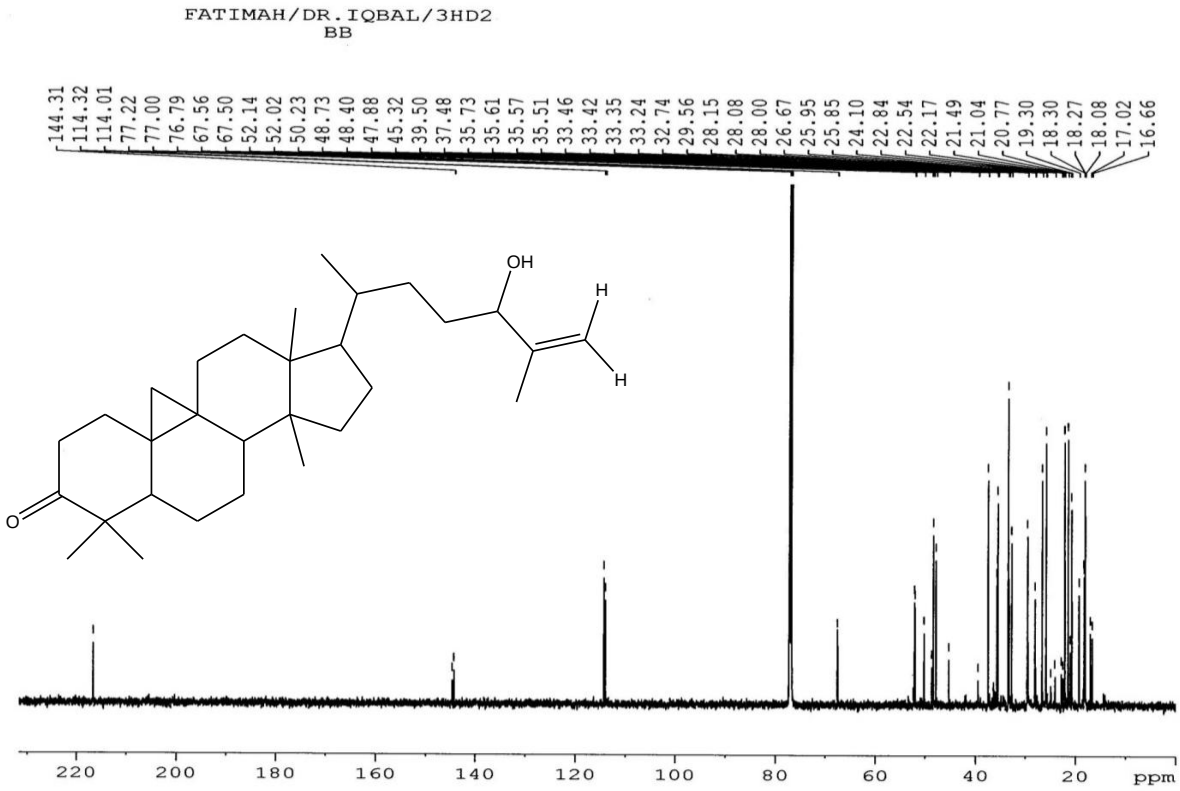


Figure 4.32: ^{13}C NMR Spectrum of Compound 2TCHD-3

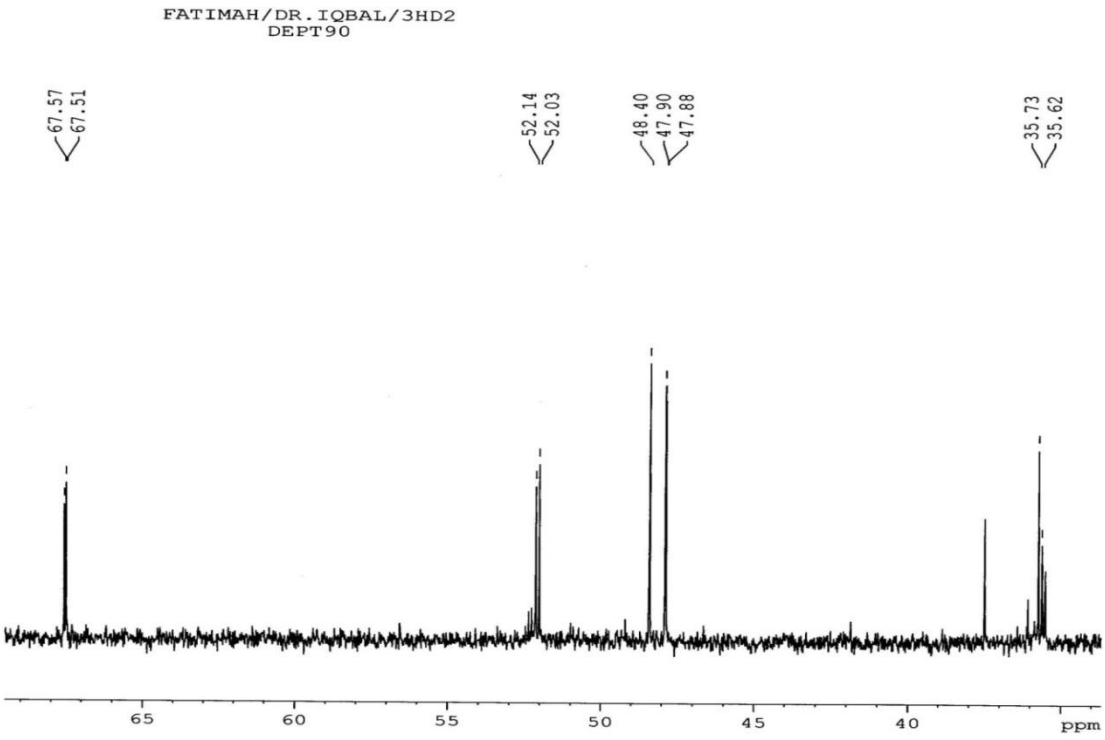


Figure 4.33: Dept 90 NMR Spectrum of Compound 2TCHD-3

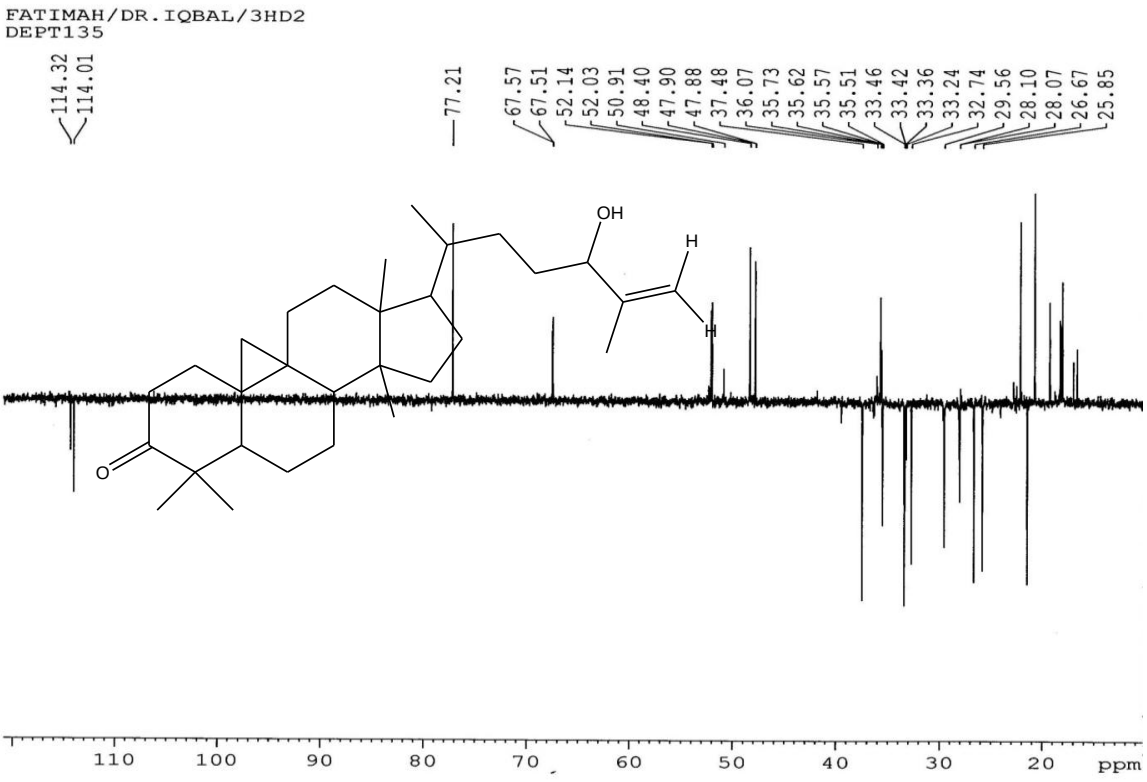


Figure 4.34: Dept 135 NMR Spectrum of Compound 2TCHD-3

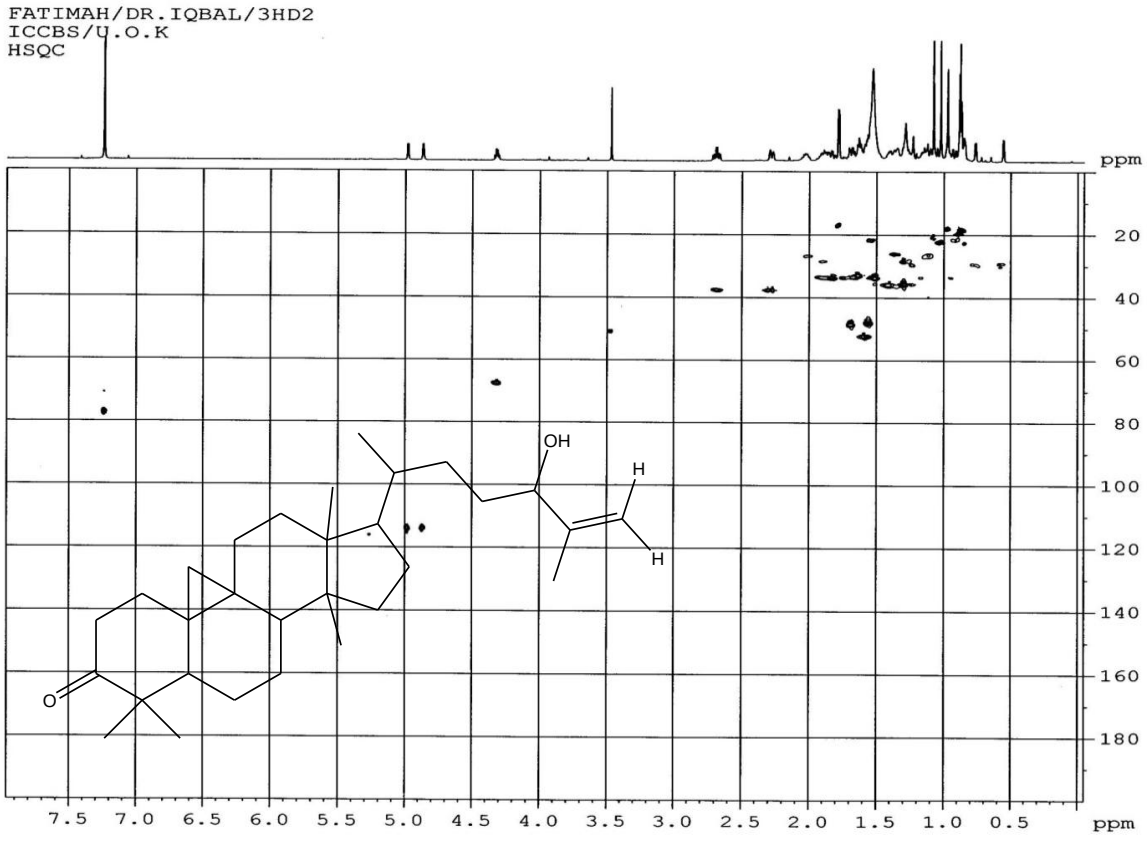


Figure 4.35: HSQC NMR Spectrum of Compound 2TCHD-3

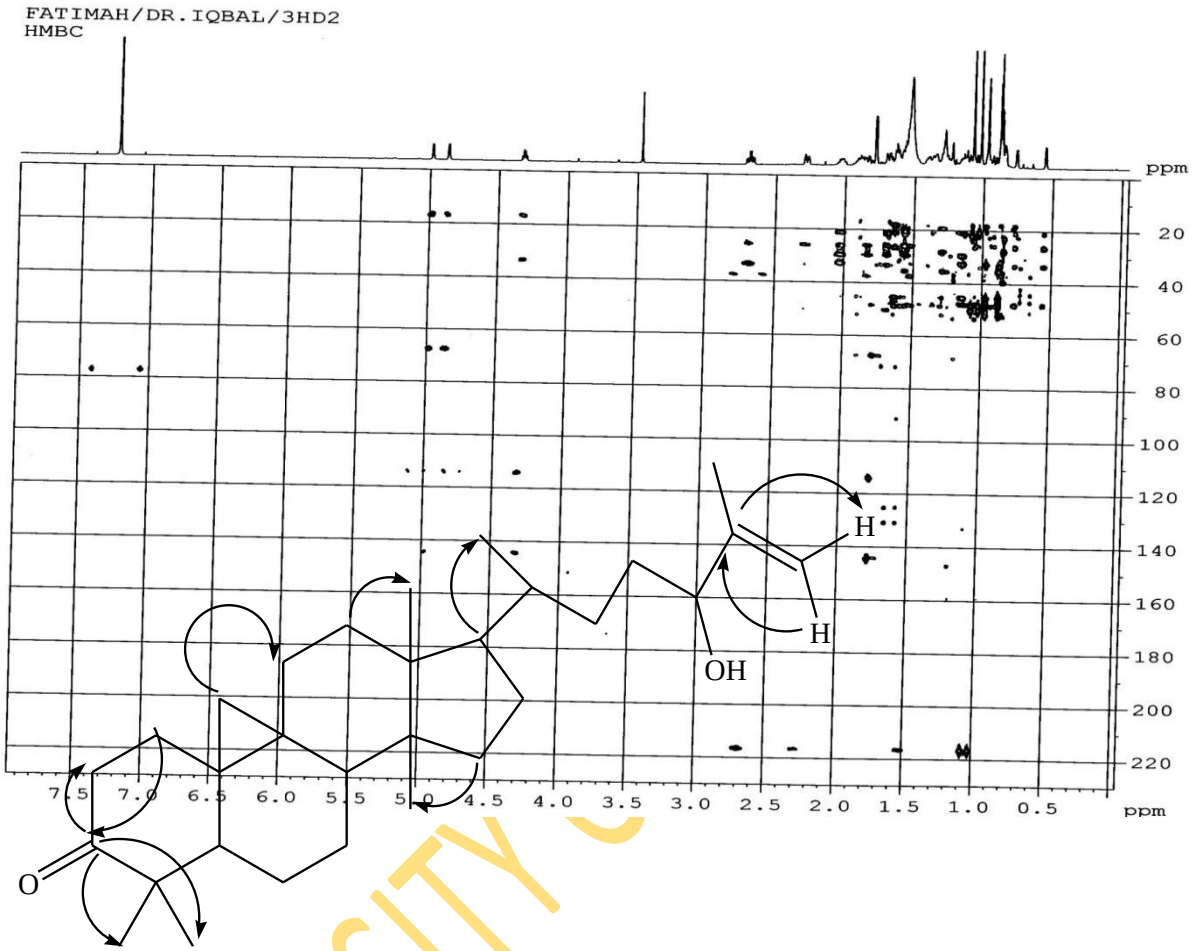


Figure 4.36: HMBC NMR Spectrum of Compound 2TCHD-3

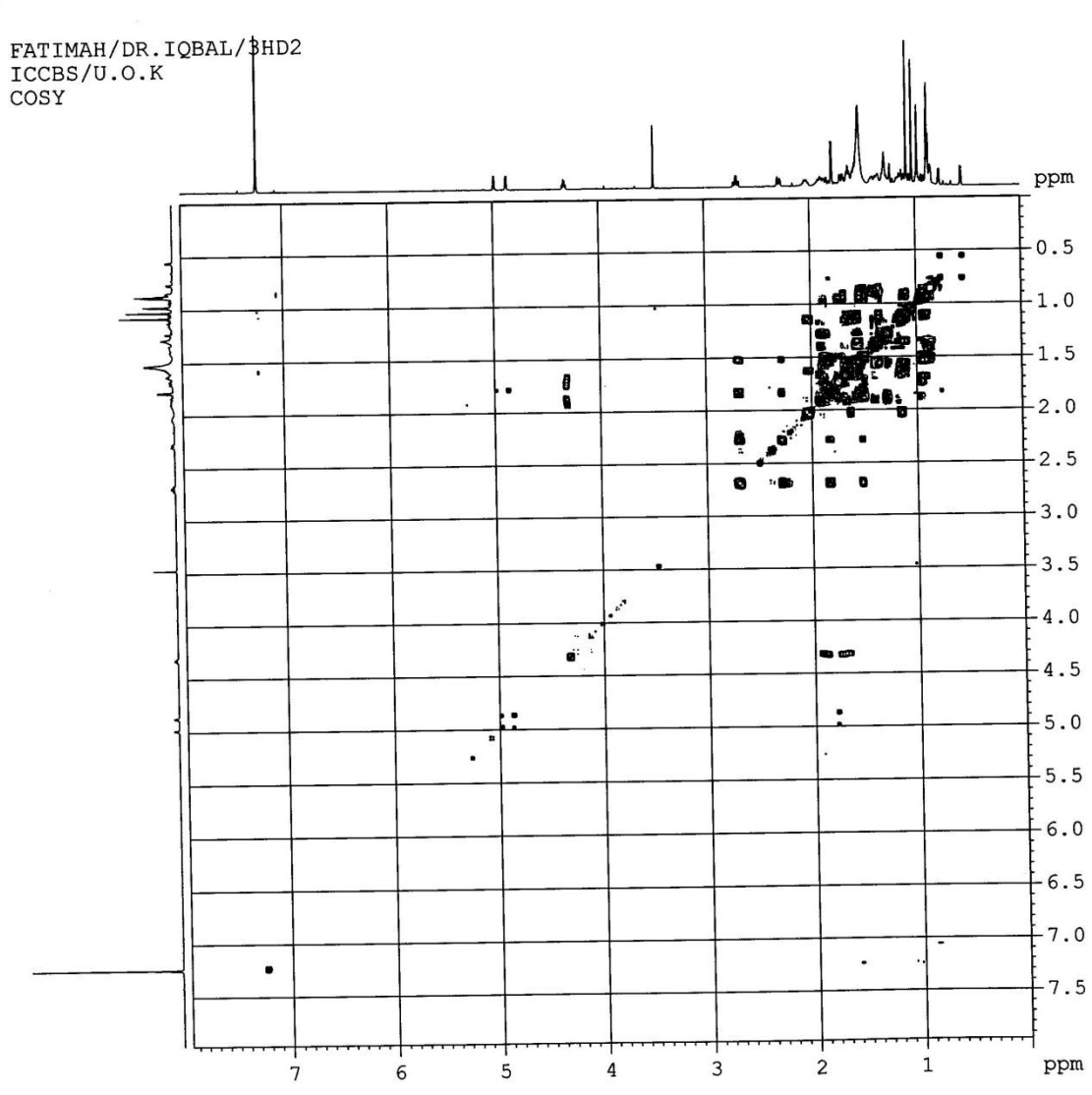


Figure 4.37: COSY Spectrum of Compound 2TCHD-3

Table 4.10: ^{13}C and ^{1}H NMR data of 2TCHD-3 and 24-hydroxy-25-cycloarten-3-one

Position	δ ^{13}C ppm	DEPT	δ ^{1}H ppm	HMBC	δ ^{13}C* ppm	δ ^{1}H* ppm
1	33.5	CH_2	1.52,1.85	$H\text{-}_5$, $H\text{-}_{19}$	33.4	
2	37.5	CH_2	2.27, 2.3		37.5	
3	216.7	C		$H\text{-}_{28}$, $H\text{-}_{29}$, $H\text{-}_{2a}$, $H\text{-}_{2b}$, $H\text{-}_{1a}$	216.6	
4	50.2	C			50.2	
5	48.4	CH	1.69		48.4	
6	21.5	CH_2	0.9, 1.5		21.5	
7	28.1	CH_2	1.2		28.0	
8	47.9	CH	1.56	$H\text{-}_{30}$	47.9	
9	21.1	C		$H\text{-}_{19}$	21.1	
10	26.0	C			26.0	
11	26.7	CH_2	2.02	$H\text{-}_{19}$	26.7	
12	33.4	CH_2	1.5	$H\text{-}_{19}$	32.8	
13	45.3	C		$H\text{-}_{30}$	45.3	
14	48.7	C		$H\text{-}_{30}$	48.7	
15	35.5	CH_2	1.29,1.24	$H\text{-}_{30}$	36.0	
16	25.85	CH_2	1.36		25.8	
17	52.1	CH	1.58	$H\text{-}_{21}$, $H\text{-}_{18}$	52.2	
18	18.1	CH_3	0.96		18.1	0.97 (3H,s)
19	29.6	CH_2	0.78,0.55		29.5	0.76, 0.55
20	35.7	CH	1.29		35.5	
21	18.3	CH_3	0.87		18.3	0.87 (3H,d)
22	33.2	CH_2	1.64		31.9	
23	32.7	CH_2	1.63,1.82		31.5	
24	67.6	CH	4.34	$H\text{-}_{26}$, $H\text{-}_{27}$	76.7	4(1H,t,*J*=6.6)
25	144.3	C		$H\text{-}_{24}$, $H\text{-}_{26}$	147.5	
26	114.3	CH_2	4.87,4.98	$H\text{-}_{24}$	111.4	4.8, 4.9
27	17.0	CH_3	1.78		17.2	1.7 (3H,s)
28	22.2	CH_3	1.03		22.2	1.02 (3H,s)
29	20.8	CH_3	1.08	$H\text{-}_{28}$	20.8	1.08 (3H,s)
30	19.3	CH_3	0.88		19.3	0.88 (3H,s)
		OH	3.47			

Implied multiplicities of the carbon were determined from the DEPT experiment

*- (Chiu *et al*., 2008)

4.3.2 Spectroscopic Analysis of 72TCDE-1

Compound 72TCDE-1 (48.6 mg) with the structure in Figure **4.38** appeared as a white amorphous solid with a melting point of 110.4-112.9 ^{o}C. The IR absorption bands (Figure **4.39**) show the presence of hydroxyl (V_{OH} stretching 3346 cm^{-1}), aliphatic hydrocarbons (V_{CH} stretching 2949 and 2869 cm^{-1}), 1676 cm^{-1} ($V_{C=C}$ absorption peak); other absorption peaks includes 1460 cm^{-1} (V_{CH_2}), 1049 cm^{-1} (cycloalkane) and (V_{CO} stretching 1340 cm^{-1}) as presented on Table **4.11**. The molecular formula $C_{29}H_{50}O$ (414.2) was confirmed by EIMS fragment ions 369.2 (M-45) or loss of HO^{+}=CH-CH_3 (Figure **4.40**). Ion peak at m/z 273 is due to the formation of carbocation by β-bond cleavage of side chain leading to the loss of $C_{10}H_{23}$ that corresponds to M-143. The dehydration of fragment at m/z 273 would yield m/z 255, which on successive dealkylation would yield ions at m/z 187, 173, 159, 144, 132, 106.9, 69, 54.9, 42.9.

The signals of 1H NMR spectra showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.64 and 1.01; three methyl doublets that appeared at δ 0.81, 0.83 and 0.93; and a methyl triplet at δ 0.84 (Figure **4.41**). The 1H NMR signals at δ_H 3.53 (m) and δ_H 5.32 (t) revealed the presence of oxymethine and olefinic methine protons at C-3 and C-6 respectively. The signals at δ_C 71.79, δ_C 121.7 and δ_C 140.7 were due to the presence of oxymethine carbon at C-3 and olefinic carbons at C-5 and C-6 (Figure **4.42**).

DEPT 90 (Figure **4.45**) and DEPT 135 (Figure **4.45**) exhibited six methyls, eleven methylenes and ten methine carbons.

The HSQC spectrum (Figure **4.45**) showed that methine protons at δ_H 3.53 (1H, m, H_{-3}) and δ_H 5.32 (1H, t, H_{-6}) were bonded to carbons at δ_C 71.79 (C_{-3}) and δ_C 121.7 (C_{-6}) respectively. HMBC spectrum (Figure **4.46**) reveals the correlation of methine proton; H-6 with C-4, C-7 and C-8 while oxymethine carbon; C-3 was correlated to protons H-1, H-2 and H-4. There was correlation between the methane carbon; C-17 and H-18, H-19, H-22, H-16 and H-15. The COSY spectrum (Figure **4.47**) revealed the correlation of oxymethine proton at C-3 with methylene protons at C-2 and C-4 while olefinic methine proton at C-6 was correlated with methylene protons at C-7. Thus, the structure of 72TCDE-1 was assigned as Stigmast-5-en-3-ol (β-sitosterol) with

spectroscopic data (Figure **4.38**) that was consistent to the reported literature values (Habib *et al*., 2007; Jamal *et al*., 2009; Patra *et al.*, 2010; Chaturvedula and Prakash, 2012) as seen on Table **4.12** and was further supported by the key COSY and HMBC correlations as shown in Figures **4.46** and **4.47**.

Stigmast-5-en-3-ol as a steroid, is an important class of bioorganic molecules, widespread in plants. This compound has a long history of consumption as food or pharmaceutical products, and generally recognized as safe without undesirable side effects (Saeidnia *et al.*, 2014). It is usually used for heart disease, hypercholesterolemia, modulating the immune system, prevention of cancer, as well as for rheumatoid arthritis, tuberculosis, cervical cancer, hair loss and benign prostatic hyperplasia. Furthermore, its diverse biological and pharmacological activities (anti-inflamatory, anticancer, angiogenic, immunomodulatory, antihelminthic, antioxidant, neuroprotection and antidiabetic) have been reported (Prieto *et al.*, 2006; Loizou *et al.*, 2010; Chai *et al.*, 2008; Villasenor *et al.*, 2002; Baskar *et al.*, 2012; Shi *et al.*, 2013; Radika *et al.*, 2013).

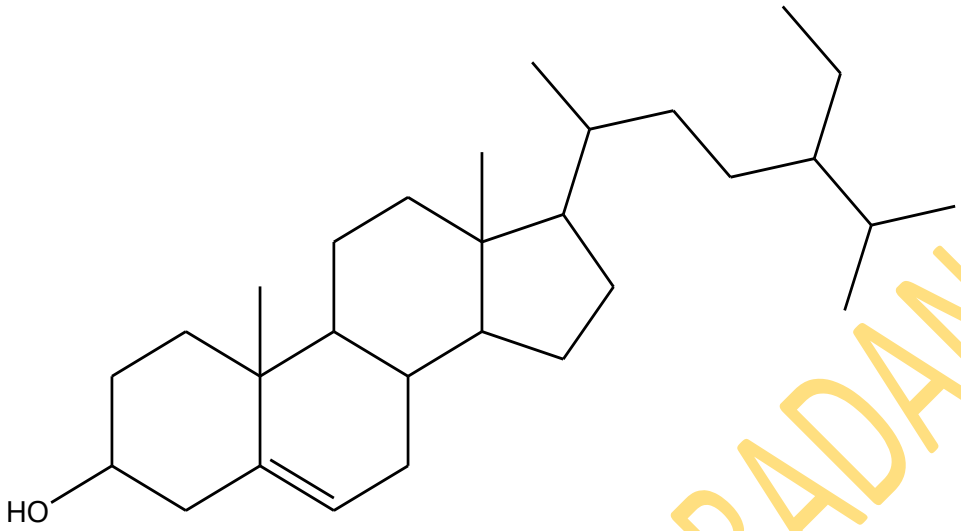


Figure 4.38: Compound 72TCDE-1 (Stigmast-5-en-3β-ol)

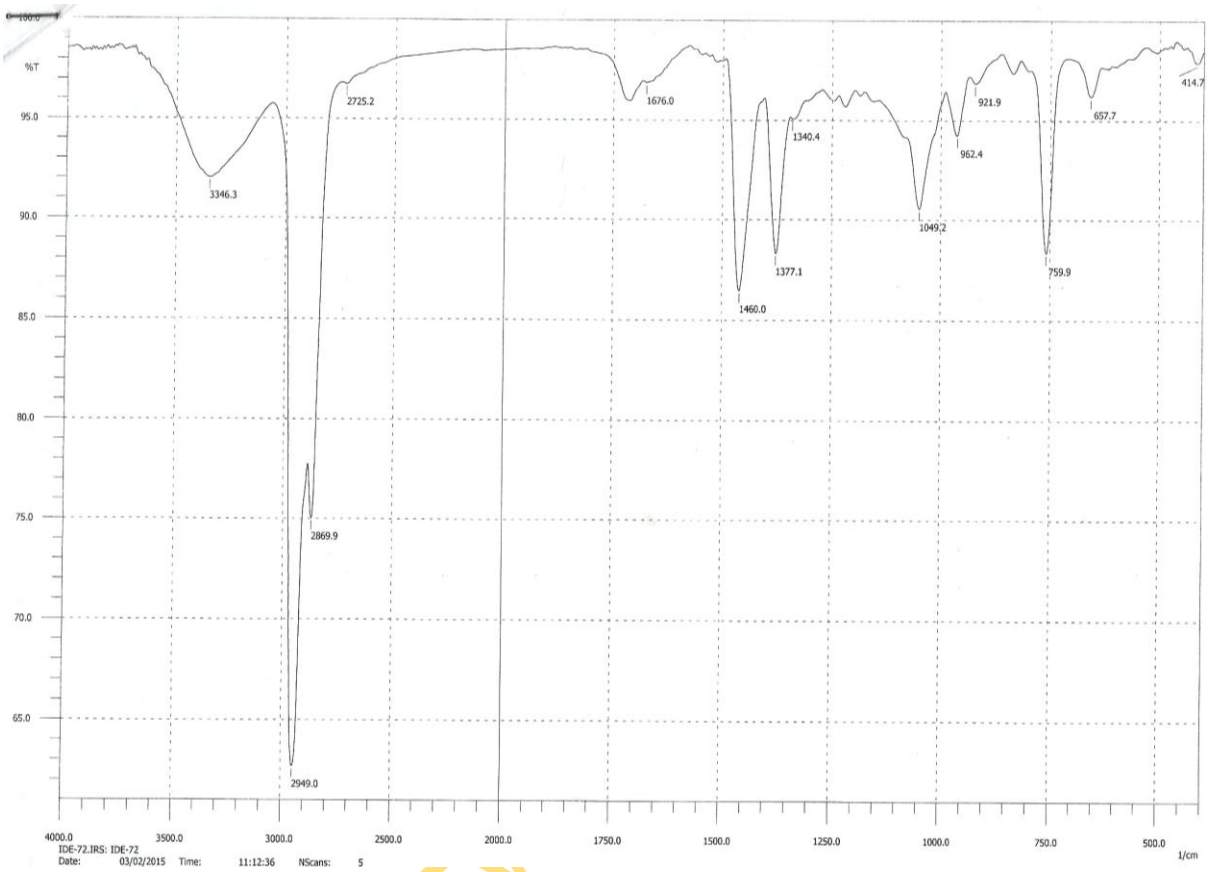


Figure 4.39: IR Spectrum of Compound 72TCDE-1

Table 4.11: Infra-Red values of Compound 72TCDE-1

Functional Group	IR values (V/cm^{-1})
C-C (Alcohol)	1340
C-H (CH_2)	1460

C=O (Ketone)	1706
C-H (Aliphatic Stretch)	2949 and 2869
O-H	3346

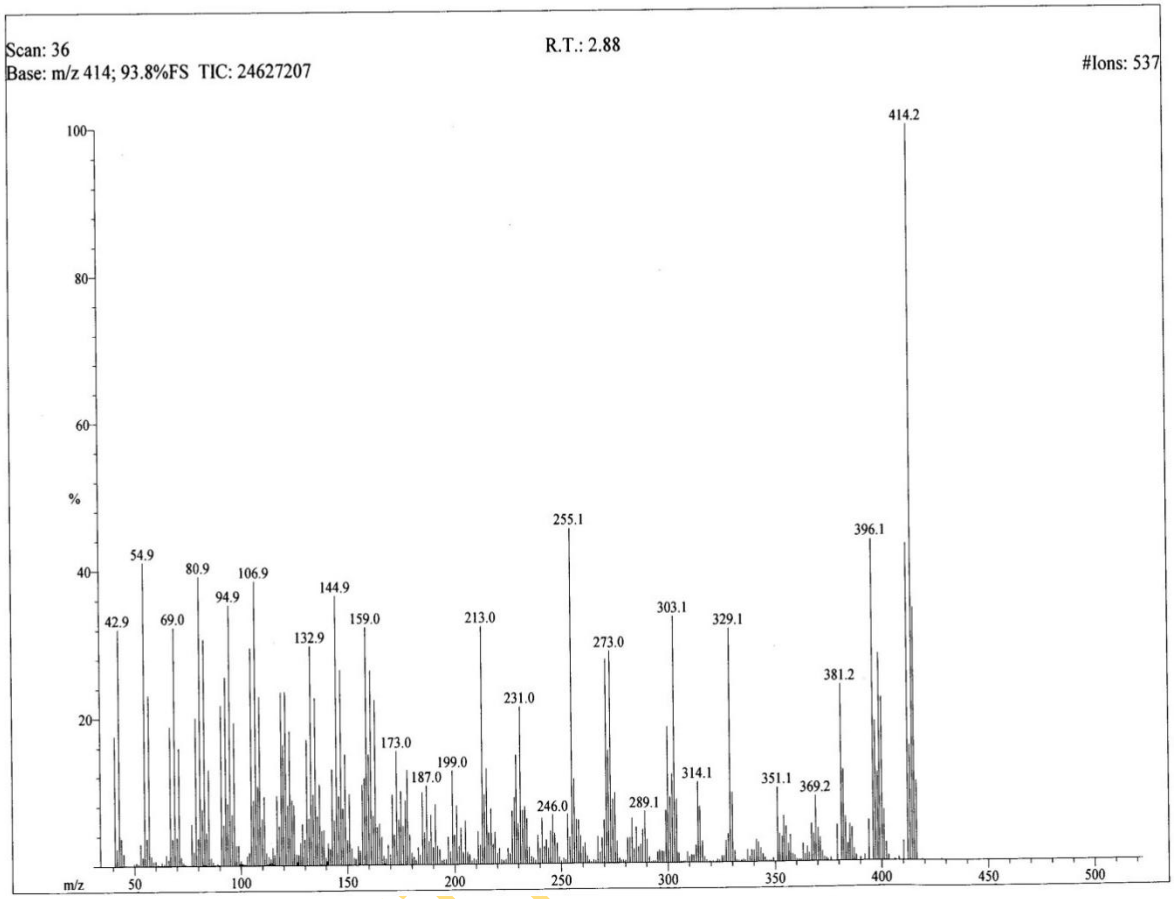


Figure 4.40: EIMS Spectrum of Compound 72TCDE-1

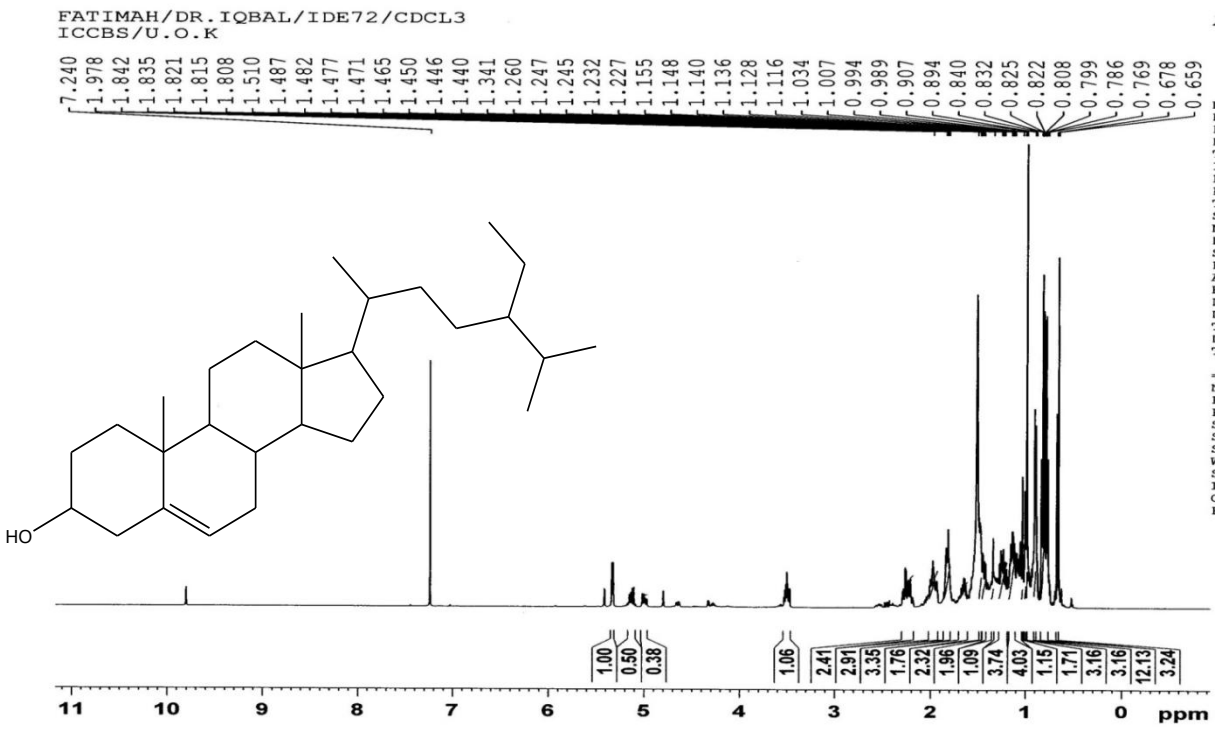


Figure 4.41: ^{1}H NMR Spectrum of Compound 72TCDE-1

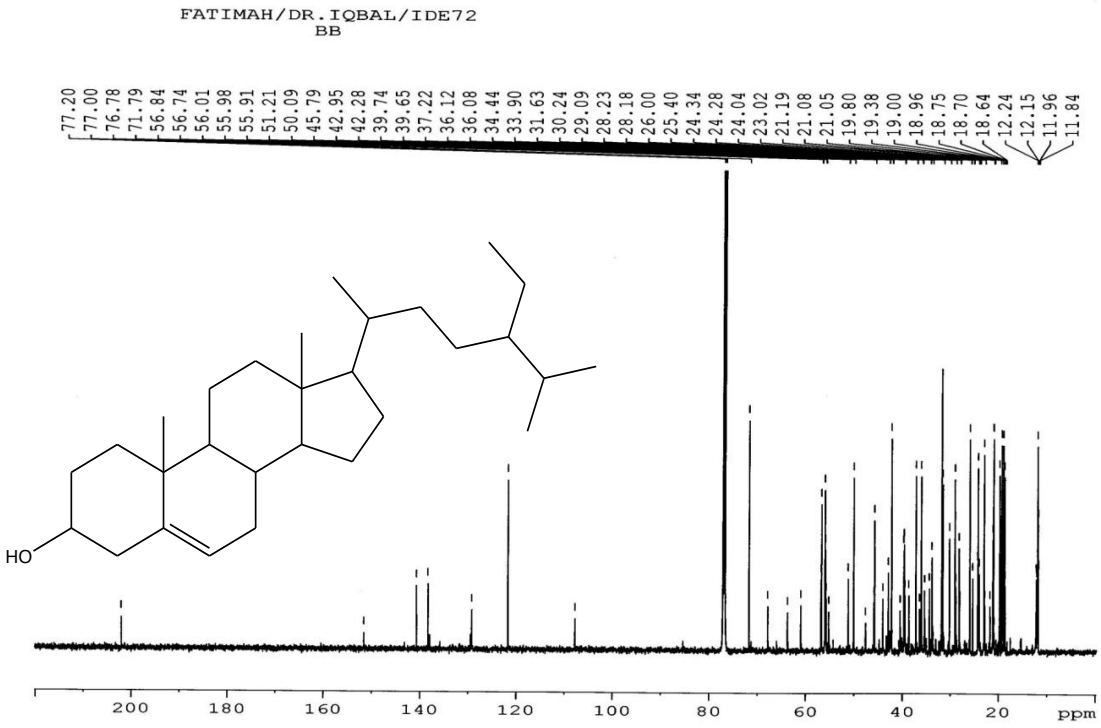


Figure 4.42: ^{13}**C NMR Spectrum of Compound 72TCDE-1**

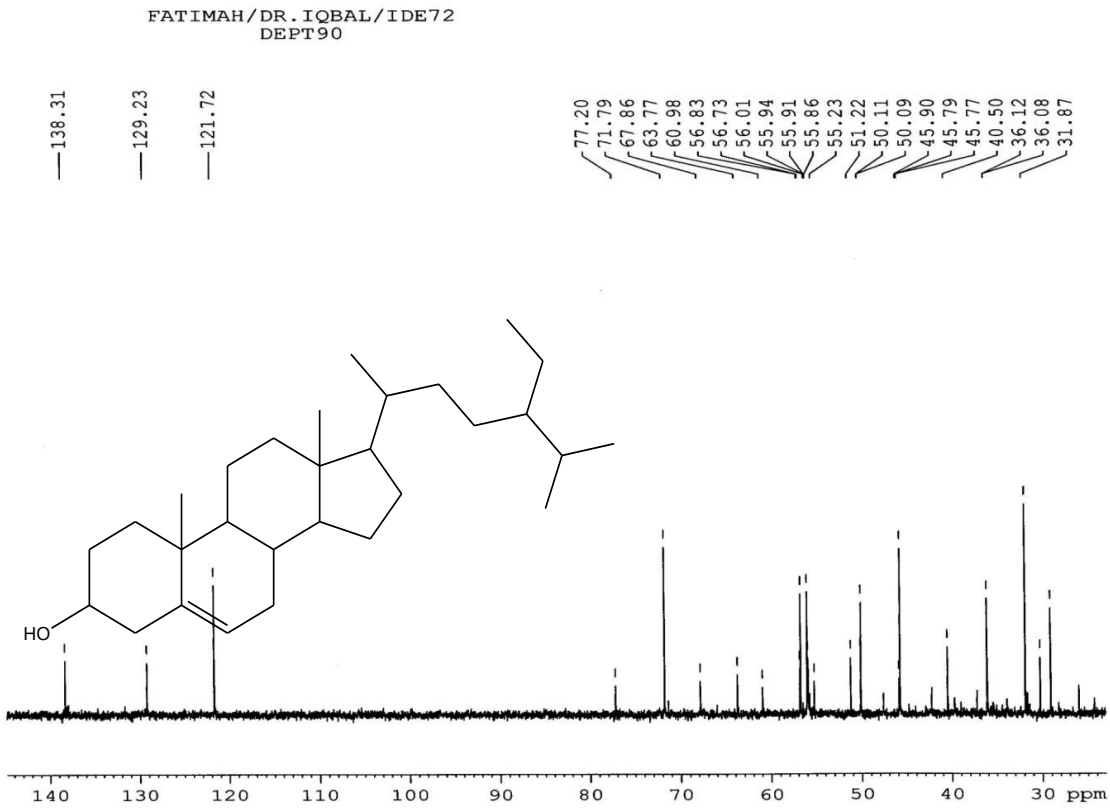


Figure 4.43: Dept 90 Spectrum of Compound 72TCDE-1

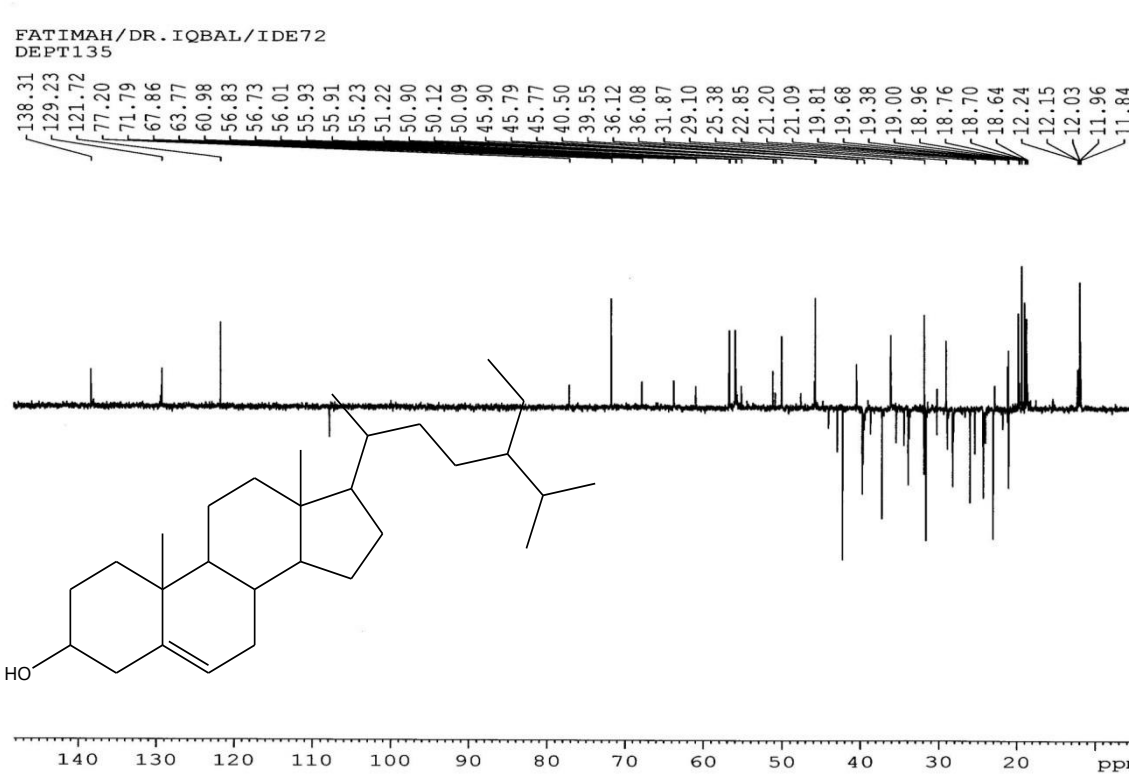


Figure 4.44: Dept 135 Spectrum of Compound 72TCDE-1

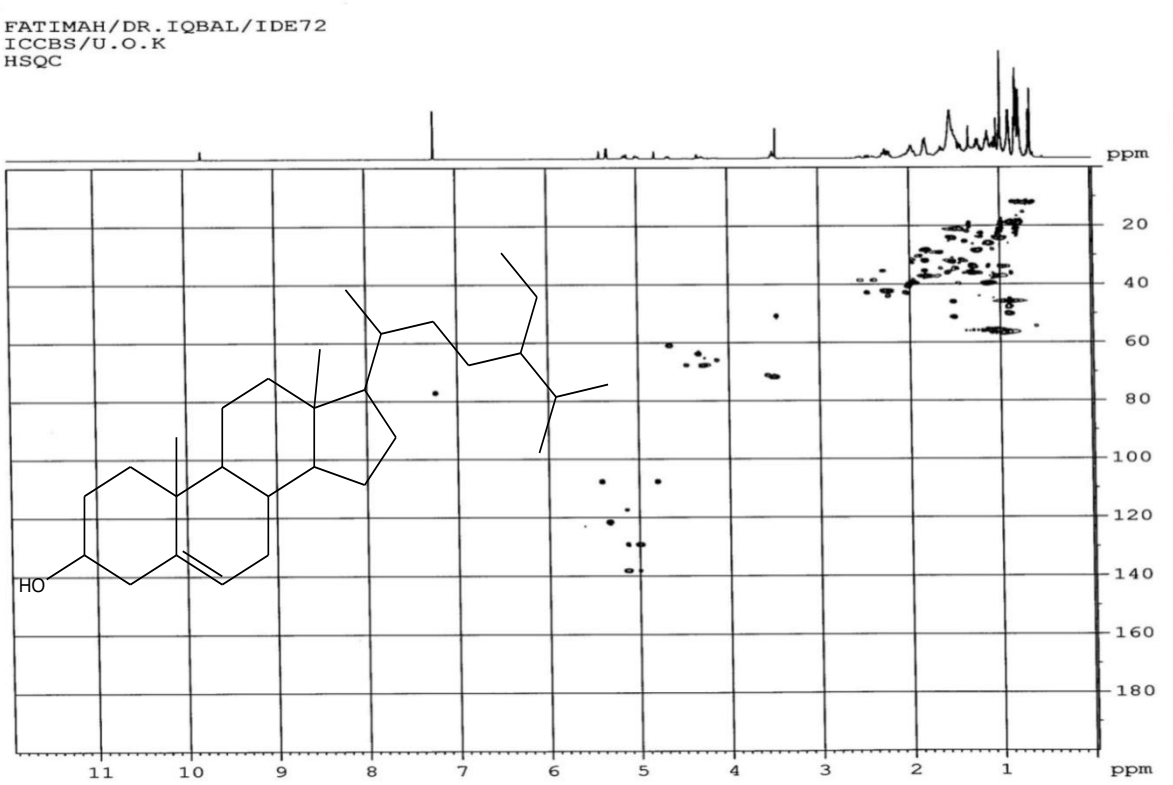


Figure 4.45: HSQC Spectrum of Compound 72TCDE-1

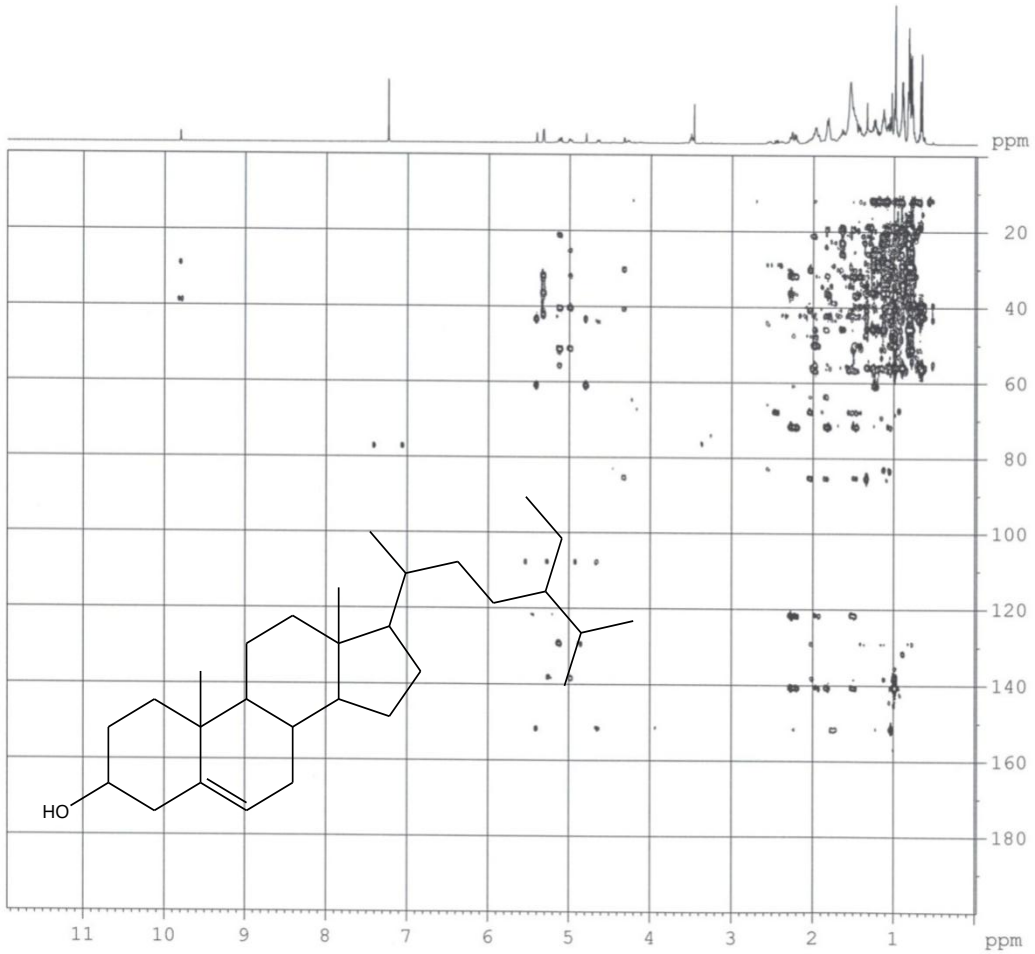


Figure 4.46: HMBC Spectrum of Compound 72TCDE-1

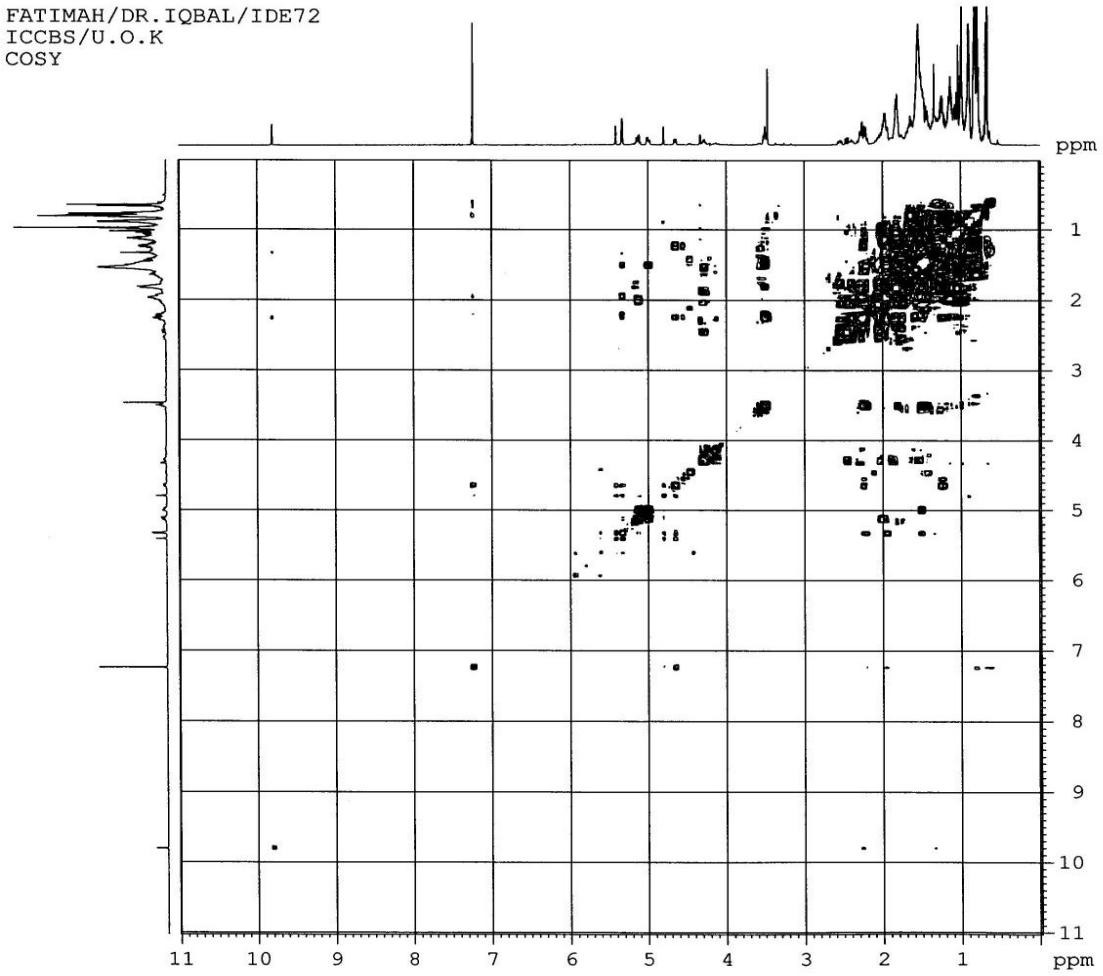


Figure 4.47: COSY Spectrum of Compound 72TCDE-1

Table 4.12: ^{13}C and ^{1}H NMR data of 72TCDE-1 and Stigmast-5-en-3-ol

Position	δ ^{13}C ppm	DEPT	δ ^{1}H ppm	δ $^{13}C^{*}$ ppm	δ $^{1}H^{*}$ ppm

1	37.22	CH_2	1.04, 1.82	37.5	1.47
2	31.64	CH_2	1.43	31.9	1.56
3	71.79	CH	3.5	72.0	3.52
4	42.28	CH_2	2.23,2.28	42.5	2.28
5	140.7	C		140.9	
6	121.7	CH	5.32	121.9	5.36
7	31.89	CH_2	1.83	32.1	2.03
8	31.87	CH	1.69	32.1	1.67
9	50.1	CH	0.9	50.3	1.48
10	36.48	C		36.7	
11	21.05	CH_2	1.37	21.3	1.52
12	39.6	CH_2	1.14	39.9	1.49
13	42.95	C		42.6	
14	56.73	CH	0.98	56.9	1.5
15	26.0	CH_2	1.14	26.3	1.6
16	28.23	CH_2	1.25, 1.84	28.5	1.84
17	55.8	CH	1.08	56.3	1.49
18	36.1	CH	1.36	36.3	1.64
19	19.38	CH_3	0.98	19.2	1.02
20	34.44	CH_2	1.48	34.2	0.88
21	25.38	CH_2	1.13	26.3	1.04
22	45.79	CH	1.52	46.1	1.5
23	23.02	CH_2	1.03	23.3	1.04
24	12.2	CH_3	0.64	12.2	0.68
25	29.1	CH	1.67	29.4	1.65
26	21.05	CH_3	0.98, 0.82	20.1	0.83
27	19.8	CH_3	1.34	19.6	0.94
28	19.0	CH_3	0.89	19.0	0.85
29	11.96	CH_3	0.87	12.0	0.88
		OH	3.42		

Implied multiplicities of the carbon were determined from the DEPT experiment
*- (Chaturvedula and Prakash, 2012)

4.3.3 Spectroscopic Analysis of 359TCDE-3

Compound 359TCDE-3 (11 mg) (Figure **4.48**) appeared as a white crystalline solid with a melting point of 144.7-146.5 ^{o}C. The IR absorption bands (Figure **4.49)** show the presence of hydroxyl (V_{OH} stretching 3346.3 cm^{-1} and V_{OH} bending 1375.2 cm^{-1}), aliphatic hydrocarbons (V_{CH} stretching 2954.7 and 2875.7 cm^{-1}) and epoxy (V_{O-O} stretching 864.1 cm^{-1}) (Table **4.13**).

The high resolution EI-MS (Figure **4.50)** showed m/z 428.43 (M^{+}) corresponding to a molecular formular $C_{28}H_{44}O_3$ (Calculated mass: 428.45278). The EI-MS spectrum m/z 428, 410, 396 correspond to $[M]^{+}$, $[M-H_2O]^{+}$ and $[M-O_2]^{+}$, respectively. The latter is characteristic of epidioxy sterols originated presumably by a Retro Diels Alder fragmentation generating a steroidal diene at positions 5 and 7 with its typical fragmentations. Peaks at m/z 253 (M+ - side chain - H_2O - O_2), 211 (fission of D ring - H_2O - O_2) and ion at m/z 337 generated by an allylic cleavage after the loss of O_2.

The ^{1}H NMR spectrum in Figure **4.51** exhibited four signals due to secondary methyl groups [δ 0.98 (3H, d, J = 6.5 Hz, H-21), 0.91 (3H, d, J = 7.0 Hz, H-28), 0.79 (3H, d, J = 7.0 Hz, H-26), 0.81 (3H, d, J = 7.0 Hz, H-27)] and two signals from tertiary methyl groups of d 0.79 (3H, s, H-18) and 0.87 (3H, s, H-19). The ^{13}C NMR spectrum in Figure **4.52** showed the presence of two disubstituted olefins [δ 130.74 (C-6), 135.4 (C-7), 135.19 (C-22), and 132.3 (C-23)], indicating that the sterol fragment of 359TCDE-3 is an ergosterol derivative. Two oxygenated quaternary carbons of δ 79.41 (C-5) and 82.14 (C-8) suggested the presence of a peroxide structure. The signal at position C3 revealed an oxygenated carbon carrying the hydroxyl substituent [δ 66.46]. The 13C NMR together with DEPT 90 (Figure **4.53**), DEPT 135 (Figure **4.54**), HSQC (Figure **4.55**), HMBC (Figure **4.56**) and COSY (Figure **4.57**) showed twenty eight carbon signal including six methyls, seven methylenes, eleven methine and four quaternary carbons. Assignments of methine, methyl and methylene protons were achieved using HSQC (Figure **4.55)** spectra. Structural connectivities were deduced using long range correlations observed in the HMBC (Figure **4.56)** spectra. Strong correlations were observed between H-2 and C-3, 4; H-4 and C-5, 3, 2; H-9 and C-5; H-19 and C-10, 5; H-22 and C-23, 24, 20; H-6 and C-5, 8; H-17 and C-14, 18, 20; H-28 and C-26, 27.

Down field signals at δ 6.48, δ 6.22 (AB quartet, J = 8, 2H, H-6, H-7) in the ^{1}H NMR spectrum revealed the presence of disubstitued double bond which were correlated with carbon signals of δ 135.39 (C-6), δ 130.68 (C-7) in HMBC spectrum.

Based on this spectroscopic data and by comparison with literature on Table **4.14** (Krzyczkowski *et al.*, 2009; Yue *et al.*, 2001; Cateni *et al.*, 2007), it was found that 359TCDE-3 corresponds to Ergosta-5α,8α-epidioxy-6,22-dien-3β-ol, more known as ergosterol peroxide and been reported for the first time in *T. cacao*.

Ergosterol peroxide has been reported to have strong antitumor, cytotoxic and anticomplementary activities (Shin *et al.*, 2001).

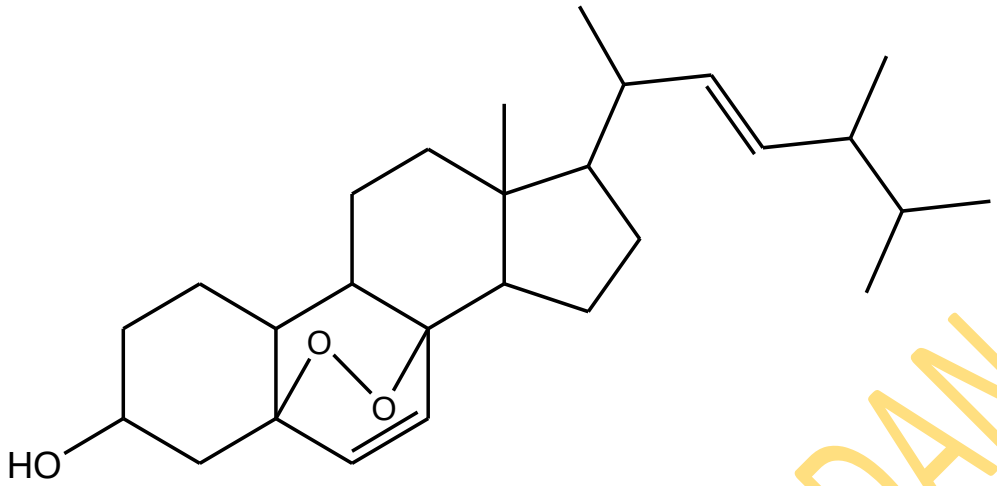


Figure 4.48: Compound 359TCDE-3 (Ergosta-5α,8α-epidioxy-6,22-dien-3β-ol) (Ergosterol peroxide)

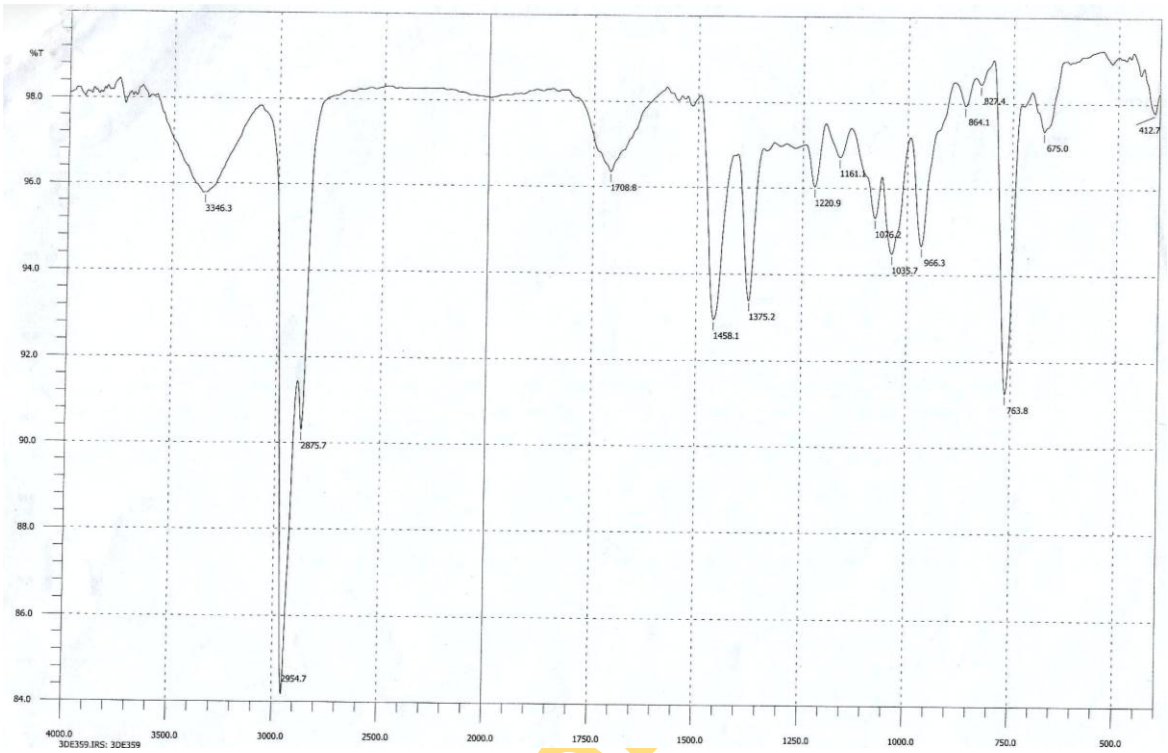


Figure 4.49: IR Spectrum of Compound 359TCDE-3

Table 4.13: IR values of Compound 359TCDE-3

Functional Group	IR values (V/cm^{-1})
O-O (Epoxy)	864
C-H (Aliphatic Stretching)	2941 and 2869
O-H (Stretching)	3346
(Bending)	1375

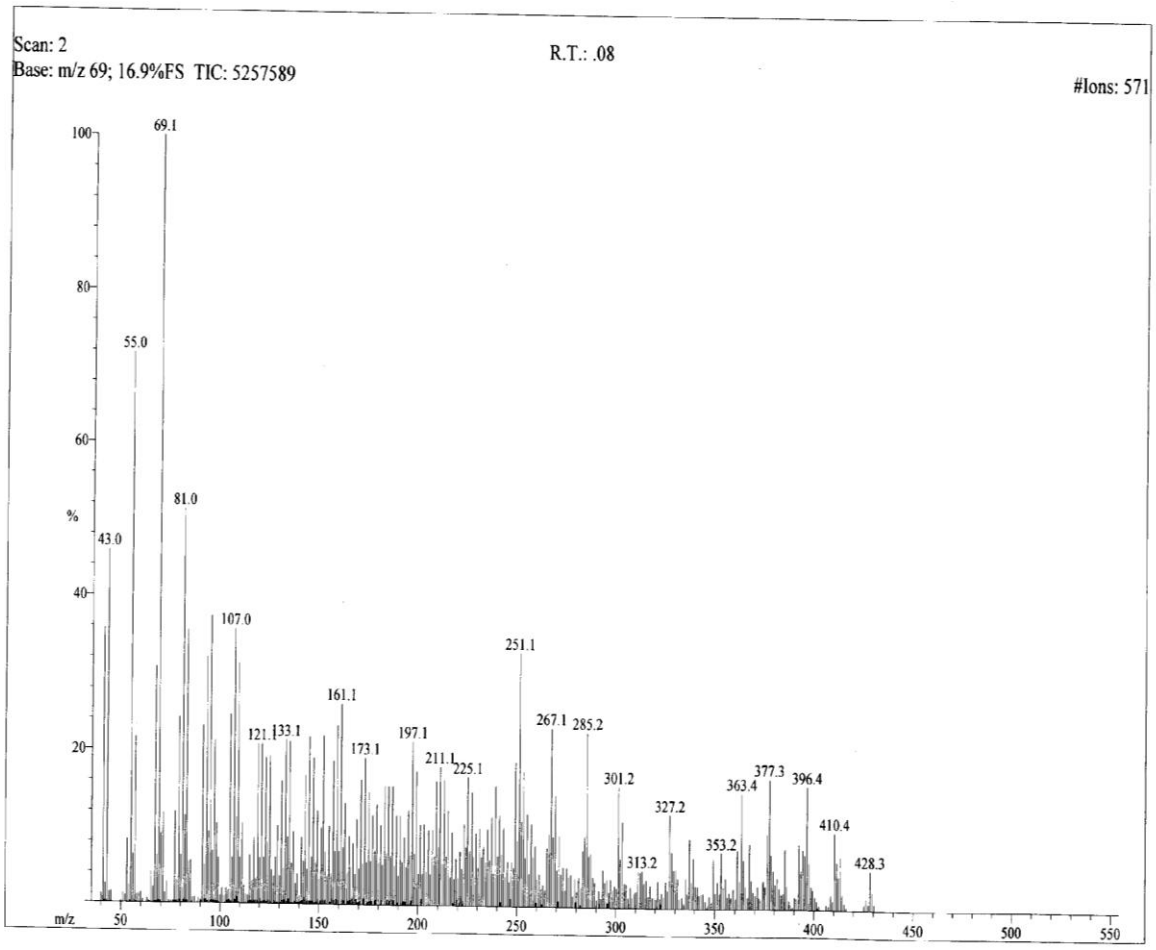


Figure 4.50: EIMS Spectrum of Compound 359TCDE-3

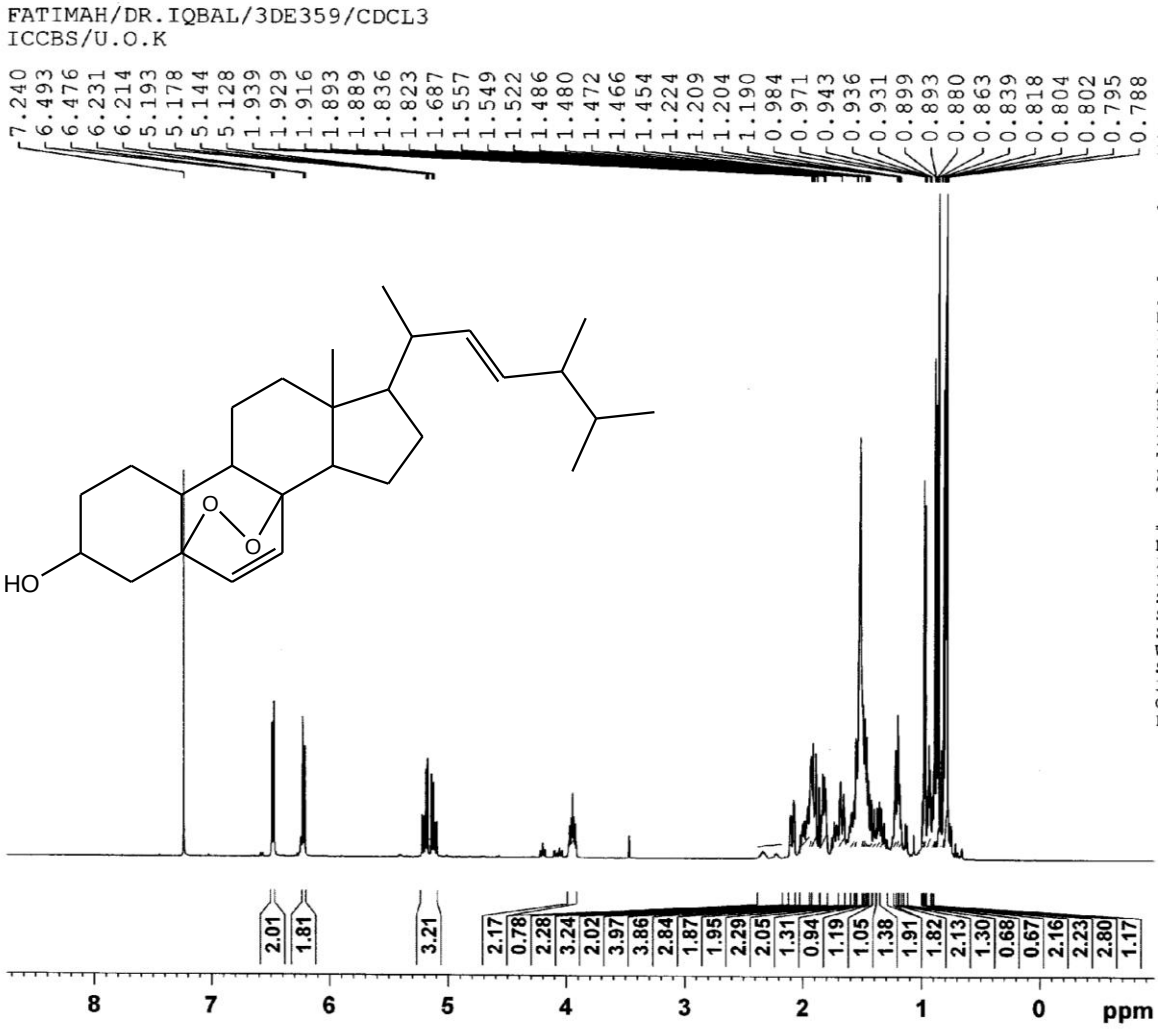


Figure 4.51: ^{1}H NMR Spectrum of Compound 359TCDE-3

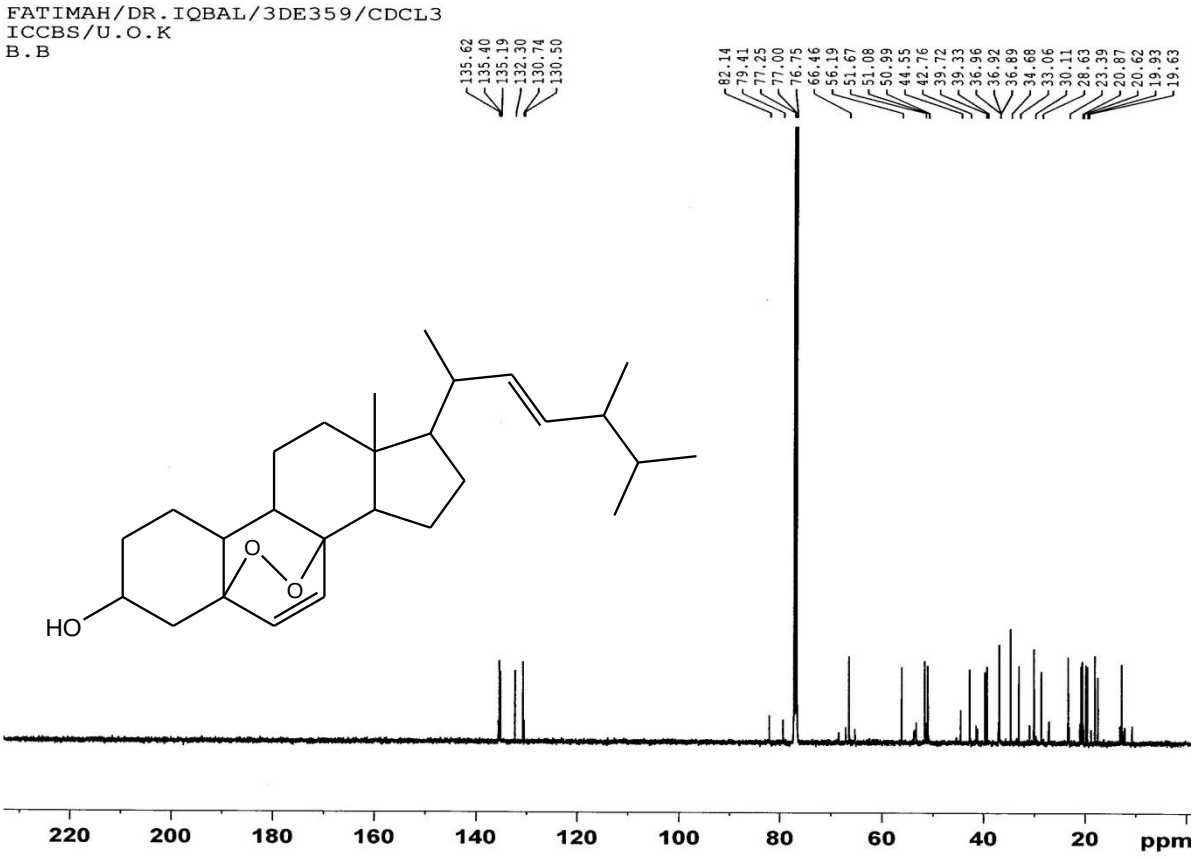


Figure 4.52: ^{13}**C NMR Spectrum of Compound 359TCDE-3**

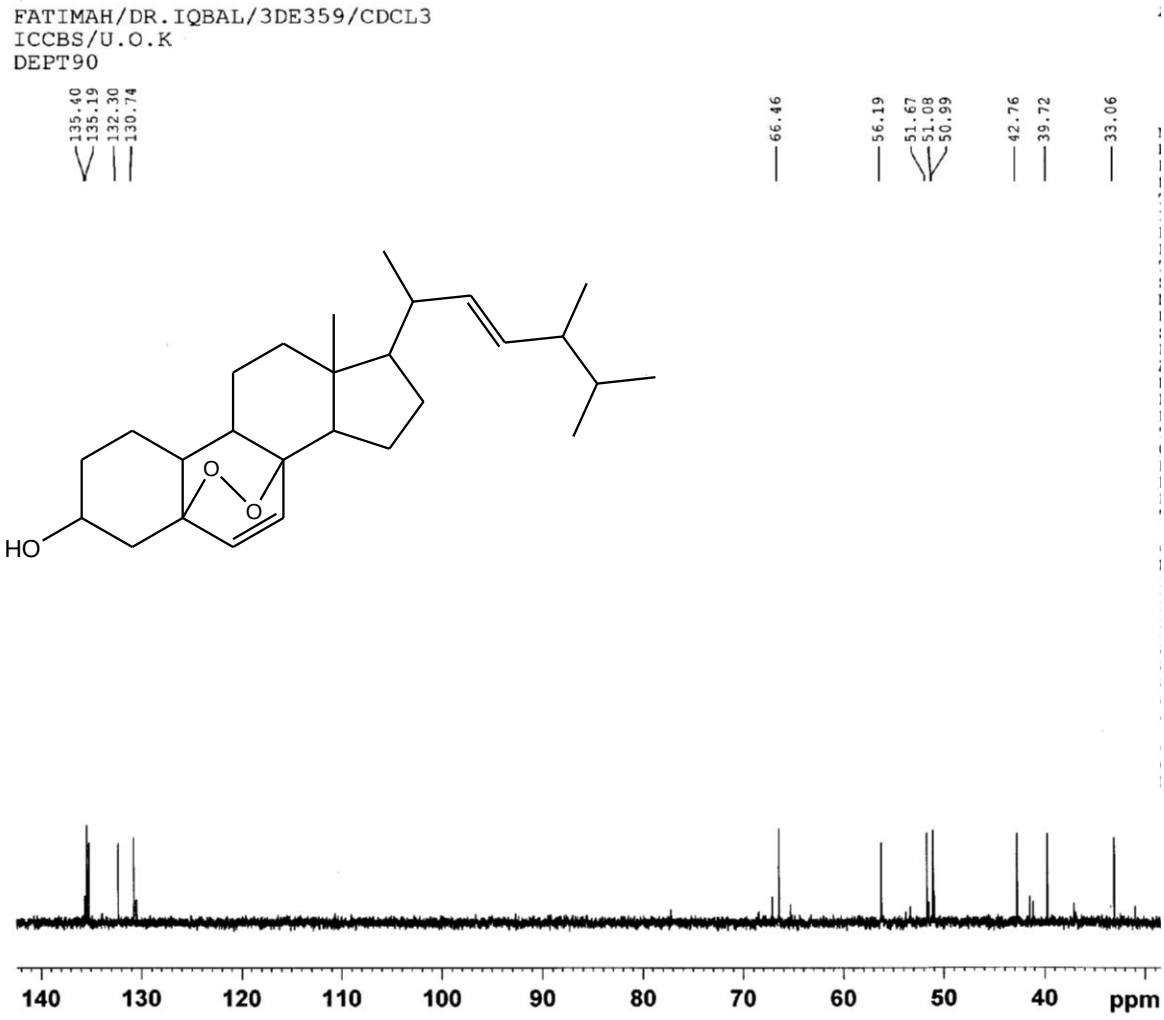


Figure 4.53: Dept 90 Spectrum of Compound 359TCDE-3

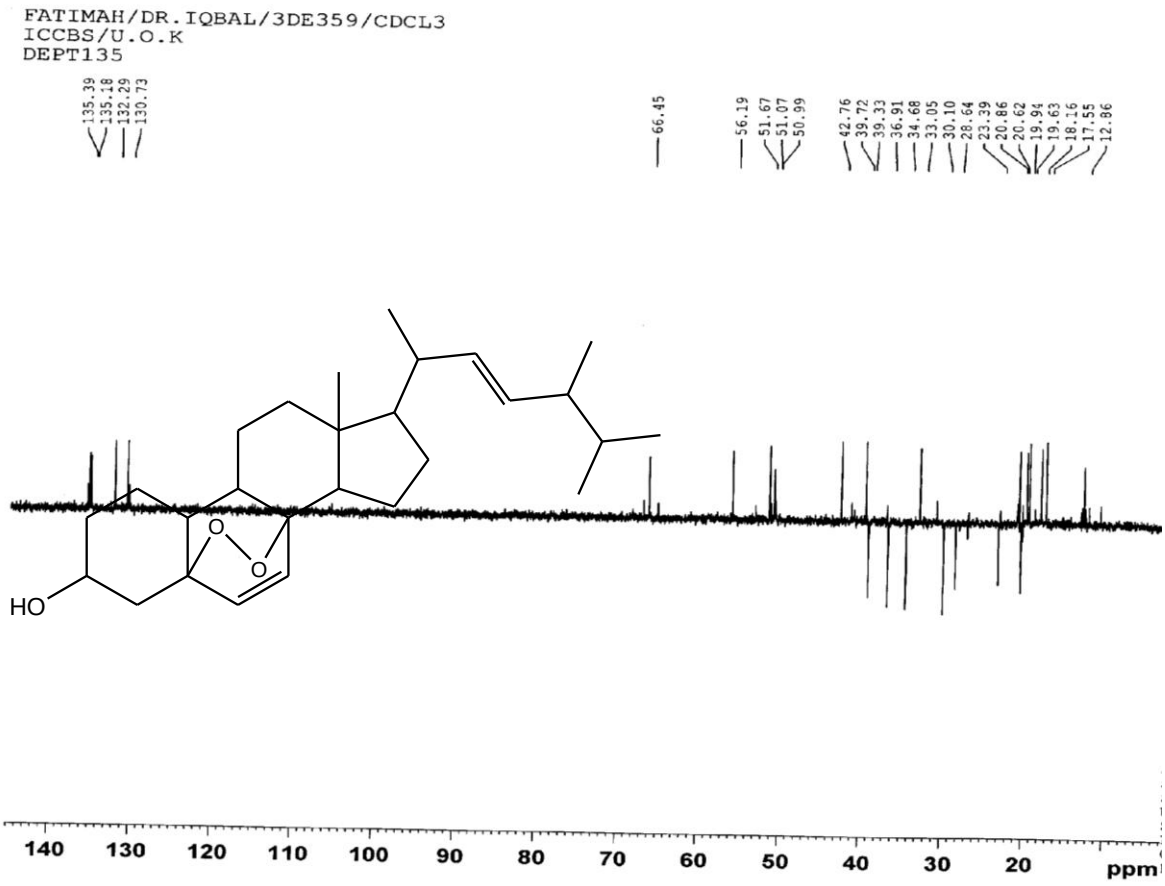


Figure 4.54: Dept 135 Spectrum of Compound 359TCDE-3

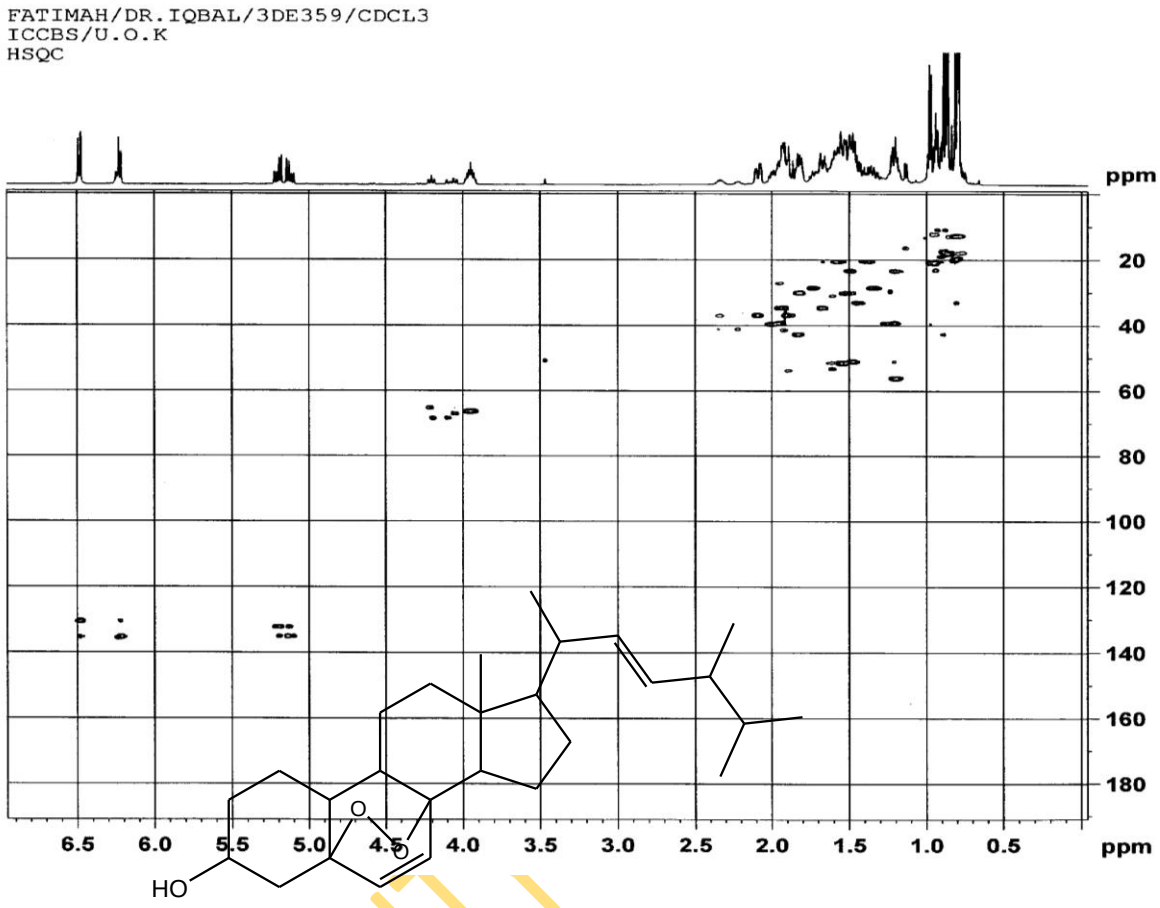


Figure 4.55: HSQC Spectrum of Compound 359TCDE-3

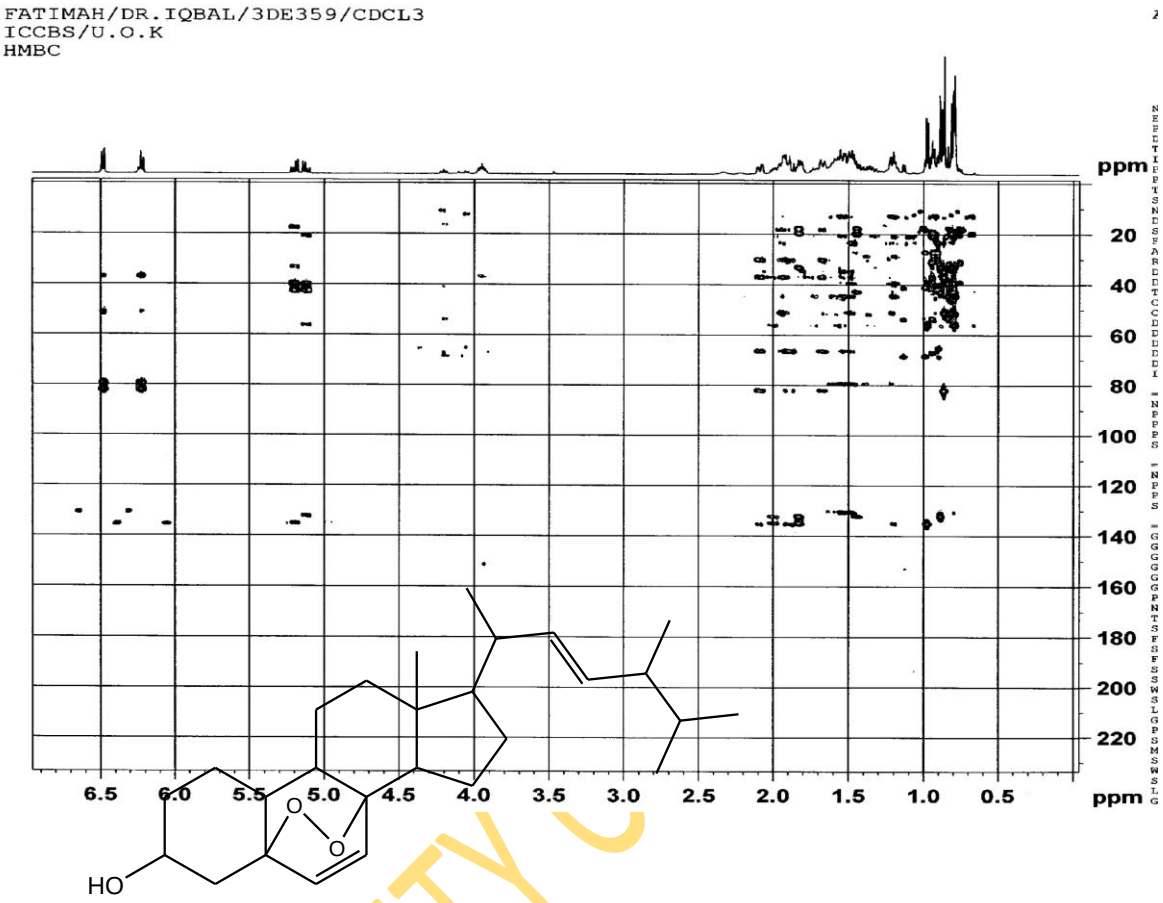


Figure 4.56: HMBC Spectrum of Compound 359TCDE-3

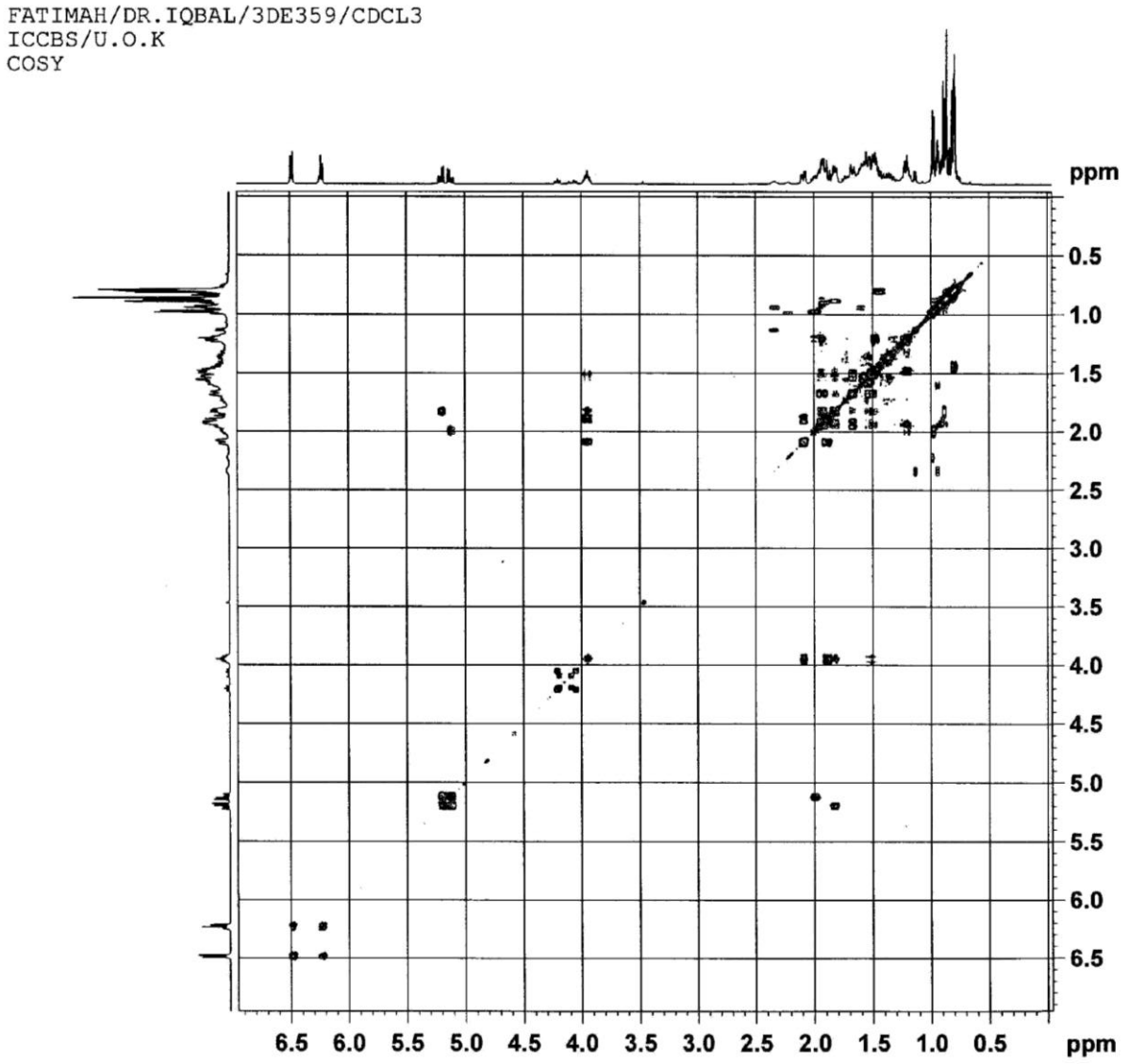


Figure 4.57: COSY Spectrum of Compound 359TCDE-3

Table 4.14: ^{13}C and ^{1}H NMR data of 359TCDE-3 and Ergosterol peroxide

Position	δ ^{13}C ppm	DEPT	δ ^{1}H ppm	δ $^{13}C^{*}$ ppm	δ $^{1}H^{*}$ ppm
1	34.68	CH_2	1.68, 1.94	34.7	1.71
2	30.10	CH_2	1.51,1.82	30.1	
3	66.46	CH	3.92	66.5	3.98
4	36.91	CH_2	1.91,2.09	36.9	
5	79.41	C		79.4	
6	130.74	CH	6.48	130.7	6.51
7	135.4	CH	6.22	135.2	6.25
8	82.14	C		82.1	
9	51.67	CH	1.55	51.1	
10	36.96	C		39.3	
11	20.62	CH_2	1.38,1.56	20.6	1.22,1.53
12	39.33	CH_2	1.22,1.92	39.7	1.25, 1.96
13	44.55	C		44.6	
14	51.08	CH	1.48	51.7	1.57
15	23.39	CH_2	1.31,1.4	23.4	1.40,1.65
16	28.64	CH_2	1.33,1.74	28.6	1.35, 1.8
17	56.19	CH	1.2	56.2	1.24
18	12.86	CH_3	0.79	12.9	0.83
19	18.16	CH_3	0.87	18.2	0.89
20	39.72	CH	2.0	36.9	2.03
21	20.86	CH_3	0.98	20.9	1.0
22	135.19	CH	5.13	135.4	5.15
23	132.3	CH	5.18	132.3	5.22
24	42.76	CH	1.83	42.8	1.85
25	33.06	CH_3	1.45	53.1	1.50
26	19.63	CH	0.79	19.6	0.82
27	19.94	CH_3	0.81	19.9	0.84
28	17.55	CH_3	0.91	17.6	0.91

Implied multiplicities of the carbon were determined from the DEPT experiment
*- (Krzyczkowski *et al*., 2009)

4.4 Biological Activity of Essential Oils

In a bid to determine the possible therapeutic value of the analysed 27 (twenty–seven) essential oils, the antibacterial, antioxidant and insecticidal activities were evaluated. The antibacterial assay was carried out on selected Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri* and *Salmonella typhi*) known as causative agents for various infectious diseases using Alamar Blue Assay. The antioxidant assay was evaluated by the method of DPPH radical scavenging activity while the insecticidal activity was done by contact toxicity test.

4.4.1 Antibacterial Activity of *Persea americana* Essential Oil

The six (6) essential oils of *Persea americana* were not active against the *Pseudomonas aeruginosa* strain while all were active against *Staphylococcus aureus* strain with percentage inhibition of 69.93%, 69.06%, 19.84%, 14.49%, 28.88% and 10.52% for the fruit, peel, seed, leaf, stem bark and root bark oils respectively. The fruit and peel oils had 11.46% and 19.05% inhibition against *Esherichia coli* strain but were not active against *Shigella flexenari* strain. The leaf and root bark oils however had 22.04% and 14.65% inhibition against *Bacillus subtilis* strain (Table **4.15**). Only the essential oil from the seed showed activity against *Salmonella typhi* strain (16.78%).

Table 4.15: Percentage Inhibition of Essential Oils of *P. americana* Mill Plant Parts

Name of Bacteria	Percent (%) Inhibition						
	Drug	**PAF**	**PAP**	**PASE**	**PAL**	**PASB**	**PARB**
Escherichia coli	72	11.46	19.05	0	0	0	0
Bacillus subtilis	76	0	0	0	22.04	0	14.65
Shigella flexenari	65	0	0	20.87	24.68	16.35	17.07
Staphylococcus aureus	79	69.93	69.06	19.84	14.49	28.88	10.52
Pseudomonas aeruginosa	80	0	0	0	0	0	0
Salmonella typhi	70	0	0	16.78	0	0	0

Key: Drug- Ampicillin, PAF- *P. americana* Fruit, PAP- *P. americana* Peel, PASE- *P. americana* Seed, PAL- *P. americana* Leaf, PASB- *P. americana* Stem Bark, PARB- *P. americana* Root Bark

4.4.2 Antibacterial Activity of *Carica papaya* Essential Oil

The eight (8) essential oils of *Carica papaya* were not active against the *Pseudomonas aeruginosa* strain while only the root bark essential oil was active against *Esherichia coli* strain (75.08%) and only the essential oil from the seed showed activity against *Salmonella typhi* strain (27.58%). The leaf oil was inactive against all the tested bacteria strains. The fruit, peel, root, stem bark and stem essential oils showed moderate percentage inhibition (13.82%-30.69%) against *Bacillus subtilis* and *Shigella flexenari* but the essential oils of the root bark, root, stem bark and stem had 67.36%, 14.73%, 13.47% and 12.10% inhibition respectively against *Staphylococcus aureus* strain while the seed oil showed 35.24% inhibition against *Bacillus subtilis* strain (Table **4.16**).

Table 4.16: Percentage Inhibition of Essential Oils of *C. papaya* Plant Parts

Name of Bacteria	Percent (%) Inhibition								
	Drug	**CPF**	**CPL**	**CPP**	**CPRB**	**CPR**	**CPSB**	**CPS**	**CPSE**
Escherichia coli	72	0	0	0	75.08	0	0	0	0
Bacillus subtilis	76	26.75	0	25.42	0	15.71	15.92	8.16	35.24
Shigella flexenari	65	28.33	0	13.82	0	30.69	16.42	26.79	0
Staphylococcus aureus	79	0	0	0	67.36	14.73	13.47	12.10	0
Pseudomonas aeruginosa	80	0	0	0	0	0	0	0	0
Salmonella typhi	70	0	0	0	0	0	0	27.58	0

Key: Drug-Ampicillin, CPF- *C. papaya* Fruit, CPL- *C. papaya* Leaf, CPP- *C. papaya* Peel, CPRB- *C. papaya* Root Bark, CPR- *C. papaya* Root CPSB- *C. papaya* Stem Bark, CPS- *C. papaya* Stem, CPSE- *C. papaya* Seed

4.4.3 Antibacterial Activity of *Ananas comosus* Essential Oil

The essential oils (3) of *Ananas comosus* were also screened against the selected bacteria and the study revealed *Esherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* strains were resistant to all the oils. *Shigella flexenari* was susceptible to all the oils; fruit, peel and shoot with 39.21%, 20.56% and 22.74% inhibiton respectively. The peel and shoot oils were also active against *Staphylococcus aureus* strain with 14.97% and 11.49% inhibition while *Bacillus subtilis* strain was susceptible to only the fruit oil with 7.36% inhibition (Table **4.17**).

Table 4.17: Percentage Inhibition of Essential Oils of *A. comosus* Plant Parts

Name of Bacteria	Percent (%) Inhibition			
	Drug	ACF	ACP	ACSH
Escherichia coli	72	0	0	0
Bacillus subtilis	76	7.36	0	0
Shigella flexenari	65	39.21	20.56	22.74
Staphylococcus aureus	79	0	14.97	11.49
Pseudomonas aeruginosa	80	0	0	0
Salmonella typhi	70	0	0	0

Key: Drug- Ampicillin, ACF- *A. comosus* Fruit, ACP- *A. comosus* Peel, ACSH- *A. comosus* Shoot

4.4.4 Antibacterial Activity of *Theobroma cacao* Essential Oil

The essential oils (4) of *Theobroma cacao* L. were also screened against the selected bacteria and the study revealed the non active nature of the essential oil from the seed against all the test bacteria strains while the leaf oil showed a high percentage inhibition (78.59%) against *Escherichia coli* strain (Table **4.18**). *Bacillus subtilis*, *Shigella flexenari*, *Pseudomonas aeruginosa* and *Salmonella typhi* strains were resistant to all the oils. *Staphylococcus aureus* strain was however susceptible to the stem bark and pod oil at 37.68% and 70.12% inhibition respectively. The stem bark and pod oils were also active against *Esherichia coli* strain with 28.95% and 31.73% inhibition.

Table 4.18: Percentage Inhibition of Essential Oils of *T. cacao* Linn Plant Parts

Name of Bacteria	Percent (%) Inhibition				
	Drug	**TCL**	**TCSB**	**TCP**	**TCSE**
Escherichia coli	72	78.59	28.95	31.73	0
Bacillus subtilis	76	0	0	0	0
Shigella flexenari	65	0	0	0	0
Staphylococcus aureus	79	0	37.68	70.12	0
Pseudomonas aeruginosa	80	0	0	0	0
Salmonella typhi	70	0	0	0	0

Key: Drug- Ampicillin, TCL- *T. cacao* Leaf, TCSB- *T. cacao* Stem Bark, TCP- *T. cacao* Pod, TCSE- *T. cacao* Seed

4.4.5 Antibacterial Activity of *Chrysophyllum albidum* Essential Oil

The essential oils (6) of *Chrysophyllum albidum* were also screened against the selected bacteria and the study revealed the non active nature of the essential oil from the seed against all the test bacteria strains except *Staphylococcus aureus* with 70.59%. *Escherichia coli* strain was resistant to all the EOs from the different parts of this fruit tree but *Shigella flexenari* was susceptible to all the volatile oils except the seed EO. *Pseudomonas aeruginosa* and *Salmonella typhi* strains were resistant to all the oils except the seed bark (10.19%) and root bark (9.79%) oils respectively (Table **4.19**).

Table 4.19: Percentage Inhibition of Essential Oils of *C. albidum* Plant Parts

Name of Bacteria	Percent (%) Inhibition						
	Drug	**CAFB**	**CARB**	**CASB**	**CASeB**	**CAL**	**CASE**
Escherichia coli	72	0	0	0	0	0	0
Bacillus subtilis	76	8.66	28.39	0	15.94	0	0
Shigella flexenari	65	21.14	35.59	9.61	6.35	13.81	0
Staphylococcus aureus	79	16.41	10.48	0	0	6.80	70.59
Pseudomonas aeruginosa	80	0	0	0	10.19	0	0
Salmonella typhi	70	0	9.79	0	0	0	0

Key: Drug- Ampicillin, CAFB- *C. albidum* Fruit Bark, CARB- *C. albidum* Root Bark, CASB- *C. albidum* Stem Bark, CASeB- *C. albidum* Seed Bark, CAL- *C. albidum* Leaf, CASE- *C. albidum* Seed

4.4.6 Comparison of the Antibacterial Activity of the Essential Oils

Researchers have reported that Gram positive bacteria are more susceptible to essential oils than Gram negative bacteria (Mann *et al.*, 2000) but in this study the essential oils did not show any preferential activity against the Gram positive and Gram negative bacteria used for the study. The oils, however, showed poor antibacterial activity against *P. aeruginosa* and *S. typhi* (both Gram negative bacteria) with percentage inhibition less than 30%. *P. aeruginosa* has been reported to be a well known antibiotic resistant Gram-negative bacterium which is generally less sensitive to the actions of plants essential oils (Boussaada *et al.*, 2008) but was sensitive to the essential oil of only *C. albidum* seed bark with 10.19 % inhibition while *S. typhi* was susceptible to only *C. albidum* root bark, *C. papaya* stem and *P. americana* seed volatile oils with 9.79%, 27.58% and 16.78% inhibition respectively. Of all the essential oils investigated for antibacterial activity against *E. coli*, *C. papaya* root bark and *T. cacao* leaf showed the most favourable efficacy with 75.08% and 78.59% inhibition respectively as against 72% inhibition of the drug. *T. cacao* stem bark, pod and *P. americana* fruit, peel oils showed relatively lower percentage inhibition (28.95%, 31.73%, 11.46% and 19.05% respectively) against *E. coli*. Twelve (12), seventeen (17) and eighteen (18) of the total twenty-seven (27) oils showed activity against *B. subtilis*, *S. flexinari* and *S. aureus* respectively. The percentage inhibition of the oils against *B. subtilis* was however low in comparison with the drug. Amongst the eighteen (18) oils that were active against *S. aureus*, it was observed that the essential oils of *C. albidum* seed, *C. papaya* root bark, *T. cacao* pod, *P. americana* fruit and peel had relatively high percentage inhibition at 70.59%, 67.36%, 70.12%, 69.93% and 69.06% respectively when compared with the drug with 79% inhibition. All the seventeen (17) oils active against *S. flexenari* had below 30% inhibition as against the standard drug's inhibition at 65%.

The notable antimicrobial variations between the oils may be attributed to the fact that the biological activity of an essential oil is linked to its chemical composition and at times to the major chemical constituents (Lawrence, 2000; Cimanga *et al.*, 2002). The major compounds in the oils might have been responsible for the antibacterial activity of the essential oils. However, it has been observed that the antibacterial effects of whole essential oils are stronger than their major components when tested individually (Lataou and Tantaoui-Elarak, 1994) and this suggests contribution of other

components other than the major compounds to the antibacterial activity of the essential oils. Some researchers have reported that a synergistic effect between the minor and major components in essential oils contributes to the antibacterial activity (Dorman and Deans, 2000; Bagamboula *et al.*, 2004; Souza *et al.*, 2007; Imelouane *et al.*, 2009). The different chemical compounds produced by plants may act synergistically during defence against attack by pathogens or during ecological adaptation to a specific habitat (Mau *et al.*, 2003; Agrawal, 2007).

Components like caryophyllene oxide, hexadecanoic acid, β- caryophyllene, linalool, β-caryophyllene, α-pinene, terpinen-4-ol and α-terpineol, 1,8 cineole, and linalool in the oils have been reported to show anti-bacterial activity (Chang *et al.*, 2000; Tzakou *et al.*, 2001; Kalemba and Kunicka, 2003; Pauli, 2004; Jovanovic *et al.*, 2005; Togashi *et al.*, 2007). There is no previous report on the antibacterial activity of the essential oils of plant parts of *C. albidum, C. papaya, T. cacao, P. americana* and *A. comosus* from any ecosystem.

4.4.7 Antioxidant Activity

The twenty-seven (27) essential oil samples were screened using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The antioxidant activity of the volatile oils was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. The percentage radical scavenging ability (% RSA) of the volatile oils were calculated based on the absorbance measurement as shown in Table **4.20**-**4.24**.

It was observed that the % RSA of essential oil of *P. americana* fruit was the highest at 42.06%. All the other parts of the plant had less than 6%. All the oils from *C. albidum* had very low % RSA with the fruit bark oil recording the highest value at 24.85% and the root bark, stem bark, seed bark, leaf and seed having below 10%. *A. comosus* shoot, peel and fruit showed 8.45%, 8.40% and 12.09% RSA. *T. cacao* seed oil gave the highest value at 38.67% RSA while the leaf, stem bark and pod oils showed 5.06, 5.25 and 4.38% RSA. The stem bark, fruit and stem oils of *C. papaya* had 11.35, 10.26 and 11.36% RSA while all other parts had less than 10% with the peel oil having the least at 2.25%. The % RSA of all the oils were lower than that of the standards used for the study. Gallic acid and n-acetyl cystein were used as standards and had 93.13 and 95.95% RSA respectively.

The observed low % RSA of the essential oils can be explained by the fact that the oils are not capable of donating hydrogen atom and the low solubility provided by the oils in the reaction medium of the assay because this test utilizes methanol or ethanol as solvent as explained by a report by Mata *et al.* (2007). Viuda-Martos *et al.* (2009) also cited these factors as the main limitation of this assay for measuring antioxidant activity of lipophilic samples like many essential oils. Despite the essential oils tested in this study not showing significant antioxidant activity, many essential oils have shown antioxidant potential. As an example there is the research conducted by Guimarães (2010), who investigated the antioxidant activity of essential oils of *Lippia sidoides*, *Alomia fastigiata*, *Ocotea odorifera*, *Mikania glauca* and *Cordia verbenacea*, and their major constituents, by the methods of the β-carotene/linoleic acid oxidation system, the formation of thiobarbituric acid reactive species (TBARS) and the reduction of the stable DPPH radical, and found that the essential oil of *L. sidoides* showed higher antioxidant activity, presenting the lowest IC_{50} values in all trials, and the antioxidant activity presented by the essential oil of *L. sidoides* was attributed to its major constituent carvacrol, which also showed high antioxidant activity when assessed in isolation (Andrade *et al.*, 2013).

The phenolic content in plants has been reported to be responsible for the antioxidant activity of some plants (Othman *et al.*, 2007). Phenolic compounds like thymol and carvacrol found in some plant essential oils have been reported to have antioxidant activity (Miguel *et al.*, 2004). Also essential oils rich in monoterpene hydrocarbons have been reported to have high antioxidant activity (Tepe *et al.*, 2005). Ruberto and Baratta (2000) investigated the antioxidant activity of 98 pure essential oil components, which represent the main classes of typical compounds of essential oils and found out that sesquiterpene hydrocarbons exerted a low, if any, antioxidant effect.

The analysis of the essential oil components in this study revealed that the oils were mainly dominated by sesquiterpenes and non-terpenes. The poor antioxidant activity of these essential oils, probably, is due to their lack of phenolic compounds and low concentrations of monoterpene hydrocarbons. However, it has been observed that correlation of the antioxidant activities of essential oils and their chemical compositions is often very complicated (Miguel, 2010). Essential oil constituents acting individually or synergistically may contribute to the antioxidant activity of the oil (Tiwari, 2001).

Table 4.20: Percentage Radical Scavenging Activity of Essential Oils of *P. americana* Mill Plant Parts

Plant Material		% Radical Scavenging Activity
PAP		5.12
PAL		3.67
PASE		1.18
PAF		42.06
PARB		2.21
PASB		2.03
Standard	GALLIC ACID	93.13
	n-ACETYL CYSTEIN	95.95

Key: PAF- *P. americana* Fruit, PAP- *P. americana* Peel, PASE- *P. americana* Seed, PAL- *P. americana* Leaf, PASB- *P. americana* Stem Bark, PARB- *P. americana* Root Bark

Table 4.21: Percentage Radical Scavenging Activity of Essential Oils of *C. papaya* Plant Parts

Plant Material		% Radical Scavenging Activity
CPRB		9.27
CPSB		11.35
CPF		10.26
CPP		2.25
CPR		2.44
CPS		11.36
CPSE		8.35
CPL		4.03
Standard	GALLIC ACID	93.13
	n-ACETYL CYSTEIN	95.95

Key: CPF- *C. papaya* Fruit, CPL- *C. papaya* Leaf,
CPP- *C. papaya* Peel, CPRB- *C. papaya* Root Bark, CPR- *C. papaya* Root
CPSB- *C. papaya* Stem Bark, CPS- *C. papaya* Stem, CPSE- *C. papaya* Seed

Table 4.22: Percentage Radical Scavenging Activity of Essential Oils of *A. comosus* Plant Parts

Plant Material		% Radical Scavenging Activity
ACSH		8.45
ACP		8.4
ACF		12.09
Standard	GALLIC ACID	93.13
	n-ACETYL CYSTEIN	95.95

Key: ACF- *A. comosus* Fruit, ACP- *A. comosus* Peel, ACSH- *A. comosus* Shoot

Table 4.23: Percentage Radical Scavenging Activity of Essential Oils of *T. cacao* L. Plant Parts

Plant Material		% Radical Scavenging Activity
TCL		5.06
TCSB		5.25
TCSE		38.67
TCP		4.38
Standard	GALLIC ACID	93.13
	n-ACETYL CYSTEIN	95.95

Key: TCL- *T. cacao* Leaf, TCSB- *T. cacao* Stem Bark, TCP- *T. cacao* Pod, TCSE- *T. cacao* Seed

Table 4.24: Percentage Radical Scavenging Activity of Essential Oils of *C. albidum* Plant Parts

Plant Material		% Radical Scavenging Activity
CARB		3.28
CASB		3.22
CASeB		2.69
CAFB		24.85
CAL		6.2
CASE		7.52
Standard	GALLIC ACID	93.13
	n-ACETYL CYSTEIN	95.95

Key: CAFB- *C. albidum* Fruit Bark, CARB- *C. albidum* Root Bark, CASB- *C. albidum* Stem Bark, CASeB- *C. albidum* Seed Bark, CAL- *C. albidum* Leaf, CASE- *C. albidum* Seed

4.4.8 Insecticidal Activity

All the oils showed no toxicity (0% mortality) against the insects except for *Chrysophyllum albidium* stem bark, *Theobroma cacao Linn.* leaf and *Ananas comosus* peel with 20% mortality against *Rhyzopertha dominica* and *Ananas comosus* peel exhibited 20% mortality of *Callosbruchus analis.* The insects were observed to be resistant to the oils used for this study based on the impregnated filter paper method used which is a form of contact toxicity. In contact toxicity stomach poisoning occurs while the insects feed on the whole grains. The weevils have to pick up the lethal dose of treatment from the essential oil to cause toxicity.

Previous studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components (Lee, 2003). The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Regnault-Roger and Hamraoui, 1995; Ahn *et al.*, 1998; Isman, 2000; Asgar, 2011). Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al.*, 2002). Due to their high volatility, they are fumigant and gaseous and might be of importance for stored-product insects (Ahn *et al.* 1998). Various monoterpenes like 1,8-cineole, linalool, α-pinene, terpinen-4-ol, and α-terpinene have been reported to show contact and fumigation toxicity to stored product pests (Papachristos *et al.*, 2004; Stamapoulos *et al.*, 2007). Therefore, the resistance of the essential oils studied for insecticidal activity may be related to the non-dominance of monoterpenes in the identified components in the oils.

The synergistic action between major and minor components of essential oils could also be responsible for the repellent action of the oils to the insects. Plant essential oils are mixtures of different major and minor components and their biological activity is generally determined by their major components or synergism/antagonism among different components (Shukla *et al.*, 2012; Tapondjou *et al.*, 2005).

CHAPTER 5

CONCLUSION

The essential oils of the fruit plants revealed diverse compounds which can be classified as hydrocarbons, monoterpenes, sesquiterpenes, diterpenes, triterpenes and non-terpenes. The non-terpenes were made up of esters, aldehydes, alcohols, fatty acids and their derivatives. The variations and similarities in the yield and compositional pattern of the essential oils may depend on factors such as plant parts utilized, storage, the plant species, drying method and the drying period. The detailed compositional pattern of the essential oils from all the plant parts of the selected fruits is being reported for the first time.

The bioassays carried out on the essential oils showed different properties with respect to the three activities of study. The antibacterial assay gave low inhibition of most of the essential oils against the test organisms. The observed antibacterial activity revealed the essential oils that can be of importance in the combat against bacteria pathogens and good sources of medicinally useful antibacterial drugs. However, *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of these essential oils as reliable antibacterial agents. Also, more detailed studies of the mechanism of actions of these oils will be of great help in utilizing their full potential in pharmaceutical, cosmetics and aromatherapy industries.

The essential oils had a relatively weak inhibition of DPPH activity. There was also non-significant insecticidal activity of the oils to the insects used for the study. The bioactivity pattern of the oils could be attributed to the interaction between compounds in the oils which usually leads to antagonistic, additive or synergestic effects. All the samples screened for secondary metabolites showed the presence of alkaloids, saponins and flavonoids with varying observation of anthocyanins, steroids, glycosides and anthraquinones. Reducing sugar was not observed in all the samples.

The isolation and characterization of the triterpenes; cycloartane (24-hydroxy-9,19-cycloanost-25-en-3-one) and a steroid (Stigmast-5-en-3β-ol) from *Theobroma cacao* Linn pod is being reported for the first time. The pharmacokinetic, structure activity relationship and toxicological profile of these isolated bioactive compounds could be carried out to explore the possibility of translating them to prescription drugs for orthodox therapies.

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