CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Medicinal plants have been in use since the beginning of human civilization and they contain substances that are useful for therapeutic purposes or which can serve as precursors for the synthesis of useful drugs.

Approximately 65,000 plant species are used in Alternative Medicine throughout the world (Schippmann *et al*., 2006). Plants and their extracts continue to provide effective treatment for diseases of all kinds and some have been found to contain compounds relevant for the management of some diseases including asthma (Ogunlesi *et al.,* 2009).

Asthma is a chronic inflammatory disorder of the airways and is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, coughing and reversible airflow limitation. The chronic inflammation that leads to the airway being hyperresponsive gives rise to a number of characteristic changes which include increased mucus secretion that leads to the formation of mucus plugs, loss of surface epithelial cells, thickening of the basement membrane and enlargement of the bronchial smooth muscle (Ashton and Crockatt, 2014). Bronchial asthma which is normaly induced by a number of agents such as allergens, infections, exercise, pollutants and certain drugs may be present in a patient throughout a lifetime.

It was Hippocrates (460-357 BC), who first described asthma and its resulting "spasms". Galen (130-201 AD), a Greco-Roman doctor, discovered that asthma was due to bronchial obstruction.

The concept, that asthma is an inflammatory disorder was firmly established in the 20th century, which also marked the advent of interventional randomized control trials (Diamant

et al., 2007). The onset of asthma for most patients is usually early in life. It can begin at any age, but most children have their first symptoms by age 5 with the pattern of disease persistence determined by recognizable risk factors. The contributions to the development of asthma include oxidative stress which is caused by overproduction of free radicals or by an insufficient antioxidant defence system (Al-Khalaf and Ramadan, 2013). Respiratory tract infections are also involved in the pathogenesis of asthma (Pelaia *et al.*, 2006). Other contributions include genetic factors such as inheritance as well as mothers being exposed to smoking during pregnancy (Bracken *et al.*, 2002), passive smoking in childhood (Miyuki and Kenichi, 2005) and dampness or dampness-related agents (Kanchongkittiphon *et al*., 2015). Specific dampness-related causal agents for exacerbation of asthma include biologic factors such as fungi, bacteria, amoeba and dust mites, or non-biologic factors such as chemicals emitted from damp materials for example formaldehyde and 2-ethyl-1-hexanol (Mendell *et al*., 2011; Norbäck *et al*., 2000). Furthermore, dampness is associated with respiratory infections which are the most common causes of exacerbation of asthma (Fisk *et al*., 2010), Jackson and Johnston, 2010).

Inflammation is the root cause of asthma, therefore anti-inflammatory medications were recommended for the treatment of the disease (US National asthma Education and Prevention, 1997). The current conventional medications for the management of severe asthma are mostly corticosteroids (Kandeel *et al*., 2013).

1.2 Statement of Problem

It is estimated that about 250,000 people die prematurely each year as a result of asthma (Bousquet *et al*., 2010). About 235 million people currently suffer from the disease (WHO, 2013) and there may be an additional 100 million persons with asthma by 2025 (The Global asthma report, 2014). Corticosteroid resistance is a major problem in patients with severe asthma (Barnes, 2012). The use of corticosteroids is also accompanied by some adverse effects such as high blood glucose, elevated blood pressure, osteoporosis, suppression of the adrenal glands, cataracts, glaucoma and low growth rate in children.

These observations necessitate the search for effective low-risk strategies such as the use of medicinal plants in the management of asthma.

The leaves of *Adansonia digitata* L., *Calotropis procera* (Aiton) W.T. Aiton, *Coix lacryma-jobi* L., *Datura metel* L., *Deinbollia pinnata* (Poir.) Schumach. & Thonn; aerial parts of *Euphorbia hirta* L. and roots of *Pterocarpus osun* Craib are used in Alternative Medicine for the management of asthma (Odugbemi, 2008) but scanty scientific research has been carried out on the chemical characterization of these plants.

1.3 Aim and objectives of Study

1.3.1 Aim of Study

The aim of the research is to extract and analyze the essential oils and methanolic extracts of the selected plants used in the management of asthma in Alternative Medicine. These extracts and fractions will be assayed for their *in vitro* antioxidant, anti-inflammatory and antibacterial activities.

1.3.2 Specific objectives

The specific objectives are as follows

a) Modification of the collection method in hydrodistillation and GC-MS analyses of the essential oil samples obtained from the listed plants out of which the plants with high percentage of known anti-inflammatory constituents will be selected for detailed study.
b) Fractionation and characterization of the 50% aqueous MeOH and hexane, dichloromethane and MeOH Soxhlet extracts of the selected plants.
c) Determination of the *in vitro* antioxidant, anti-inflammatory and antibacterial activities of the 50% MeOH and soxhlet extracts.
d) Bioactivity–guided isolation of antioxidant compounds from the plant extracts.

1.4 Significance of Study

Several plants are being used in Alternative Medicine for the management of asthma and the efficacy is different for the various plants. This study will help to know the constituents of the plants that are relevant to the management of asthma and their percentage compositions. It will also be useful to determine quantitatively their antioxidant, anti-inflammatory and antibacterial activities because these are useful in the management of asthma. These parameters will serve as a guide to the practitioners of Alternative Medicine in predicting the efficacy of the plants for the management of asthma.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

A medicinal plant is any plant, which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO, 1977).

Almost 70 percent of the prescription and Over the Counter (OTC) medicines that are being used for the management of many diseases are derived from plants and natural sources (Taur and Patil, 2011). Scientific research has isolated useful compounds from medicinal plants and as a result, numerous plants that have been abandoned are now being used in pharmaceutical preparations.

2.2 History of Medicinal Plants

The management of diseases with medicinal plant is as old as mankind itself. The use of medicinal plants was instinctive as people looked for healing of their various diseases using naturally occurring substances.

The universal role of plants in the treatment of diseases is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophy. There are Western medicines with origin in Mesopotamia and Egypt (Rooney A., 2012) and Ayurveda (Hindu) systems centred in Western Asia, the Indian subcontinent and those of the Orient (China, Japan). Oral transmission of medical information gave rise to documentation, for example, the Egyptian Papyrus Ebers which is an Egyptian medical papyrus of herbal knowledge, dating to around 1550 BC. The Ebers papyrus suggested that the treatment for asthma is with a mixture of herbs such as belladonna (*Atropa belladonna*) heated on a brick so that the sufferer could inhale the fumes. Others include baked clay tablets currently in the

British Museum, on which were written the names of contemporary drugs, parchments, herbal manuscripts, pharmacopoeias (first London Pharmacopoeia, 1618, first British Pharmacopoeia 1864) and recently upgraded to electronic storage of data. In addition to the above documented information, there is a great wealth of knowledge concerning the medicinal plants that is still transmitted orally from generation to generation by tribal societies, particularly those of tropical Africa, North and South America and the Pacific countries (Evans, 2009). Medicinal uses of plant species have progressed at an alarming rate hence there is need for the study of the plants both chemically and pharmacologically.

2.3 Constituents of Medicinal Plants

The medicinal value of plants is due to the presence of some chemical constituents in the plant tissues which produce definite physiological actions on the human body. These therapeutic agents or active principles in medicinal plants normally referred to as phytochemicals are mainly different types of secondary metabolites. The phytochemicals include: alkaloids, amino acids, flavonoids, glycosides, saponin and essential oils.

2.3.1 Alkaloids

Alkaloids are nitrogenous organic compounds which may posses some pharmacological and in many cases, medicinal activities. They are normally active at different cellular levels of organisms though they are usually very poisonous at high doses. Alkaloids can be isolated and modified by chemical, biological and bioengineering methods to enhance their properties which sometimes can be for new applications (Tadeusz, 2007).

Alkaloids can be typical (heterocyclic) or atypical (non-heterocyclic) most of which are bioactive constituents of many plants.

Typical alkaloids are "true alkaloids", which contain nitrogen in a heterocyclic ring. They are usually basic in nature. These alkaloids are highly reactive substances with biological activities even in low doses. All true alkaloids have bitter taste. There are some bioactive alkaloids which have been isolated from plants which are used in the management of diseases. Typical alkaloids can be grouped into pyrrolidine, pyridine, piperidine, tropane, quinoline, isoquinoline, phenanthrene and indole alkaloids depending on the nature of their chemical structures. Examples of bioactive true alkaloids include nicotine, quinine and yohimbine.

Nicotine, shown in Figure 1 has stimulant effects on the heart and nervous system and GC-MS analysis revealed its presence in the methanolic leaf extract of tobacco (Hossain and Salehuddin, 2013). Quinine, a quinoline alkaloid (Figure 2) is a known antimalarial which was isolated from the bark of Cinchona tree, while yohimbine (Figure 3) is an indole alkaloid which is used in the treatment of erectile dysfunction (Hozeifa, 2012).

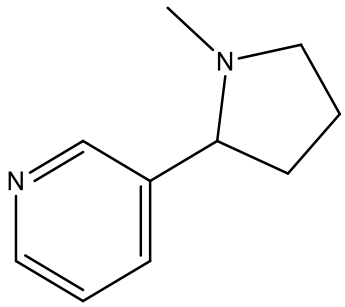


Figure 1: Structure of Nicotine [(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine]

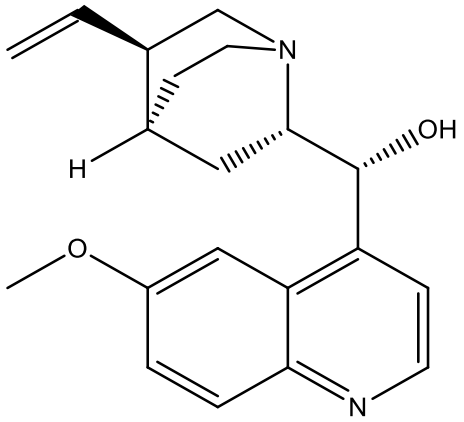


Figure 2: Structure of Quinine [(R)-[(2S,4S,5R)-5-ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl) methanol]

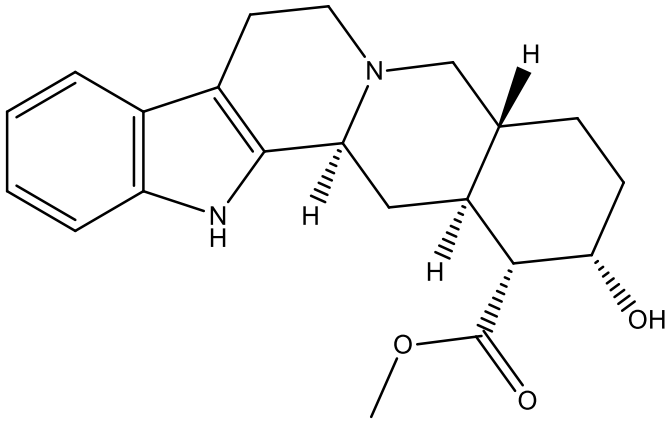


Figure 3: Structure of Yohimbine (Methyl (1S,15R,18S,19R,20S)-18-hydroxy-1,3,11,12,14,15,16, 17,18,19,20,21-dodecahydroyohimban-19-carboxylate)

Atypical alkaloids are characterized by the absence of heterocyclic ring in their molecules. They are mainly simple derivatives of phenyl ethylamine in which the nitrogen atom is located in the amino group. Few alkaloids belong to this group and the bioactive ones include: ephedrine (Figure 4), which is used in the management of asthma and allergic conditions and colchicine (Figure 5) which is used in the treatment of goitre and rheumatism (Pizzorno and Murray, 2012).

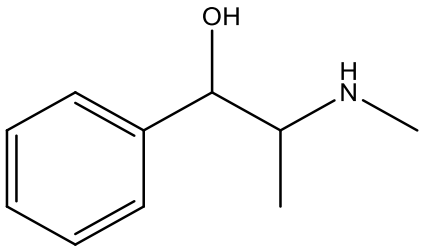


Figure 4: Structure of Ephedrine [(1R, 2S)-2-(methylamino)-1-phenylpropan-1-ol]

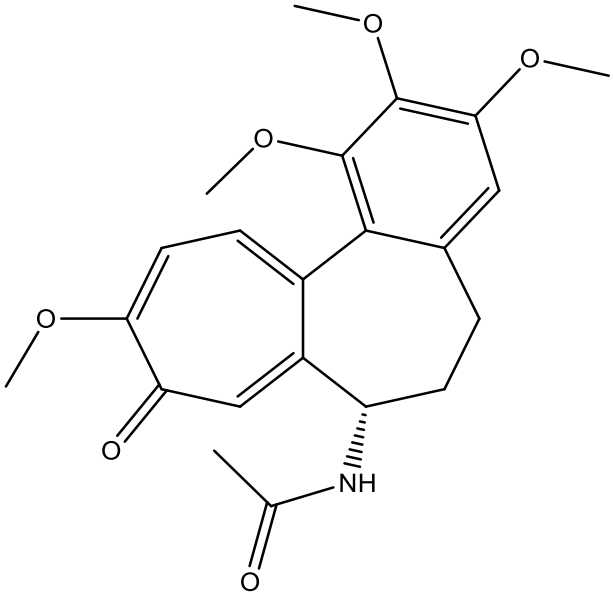


Figure 5: Structure of Colchicine (N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo[d]heptalen-7-yl]acetamide)

2.3.2 Amino Acids

These are compounds that contain one or more amino and carboxylic acid groups. Amino acids occur in plants both in the free state or as the basic units of proteins and other metabolites. They mostly contain carbon, hydrogen, oxygen and nitrogen though other atoms may be present (e.g. sulphur in cysteine, and iodine in thyroxin). The chemical structural representation of an amino aci is as shown in Figure 6.

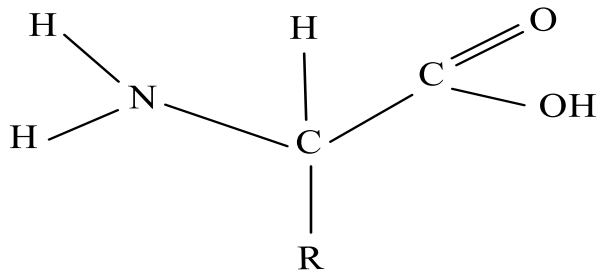


Figure 6: Structural representation of amino acid

Some of the bioactive amino acids obtained from medicinal plants include: L-Canavanine (anticancer) which is shown in Figure 7 and L-Arginine (anti-ageing) on Figure 8.

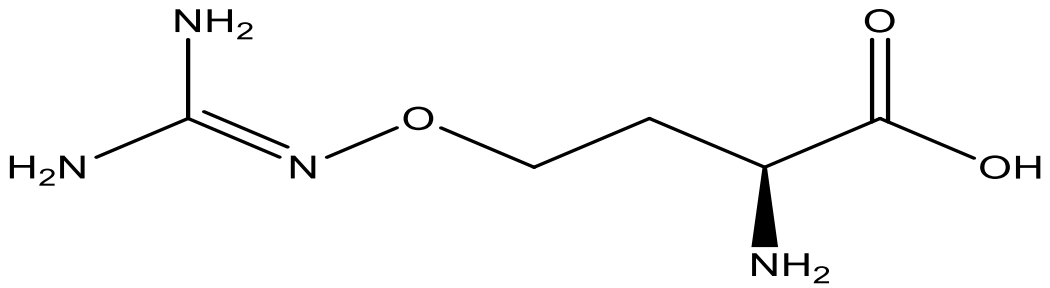


Figure 7: Structure of L-Canavanine [(S)-2-amino-4-(((diaminomethylene) amino) oxy) butanoic acid]

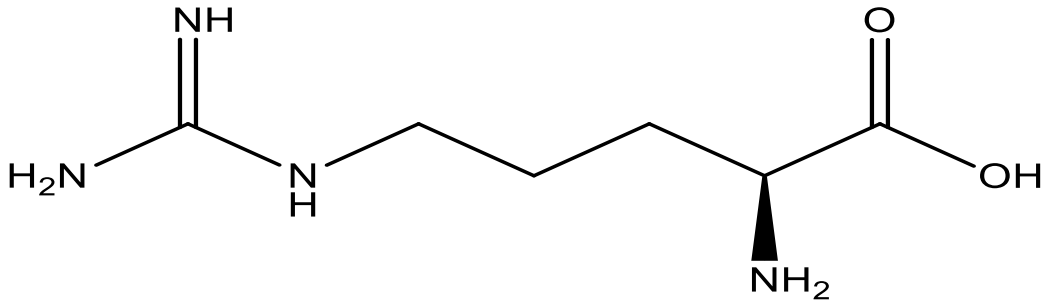


Figure 8: Structure of L-Arginine [(S)-2-amino-5-guanidinopentanoic acid]

2.3.3 Flavonoids

Flavonoids are plant pigments which have flavane (2-phenyl-benzopyrane) nucleus (Figure 9) as the basic structural unit.

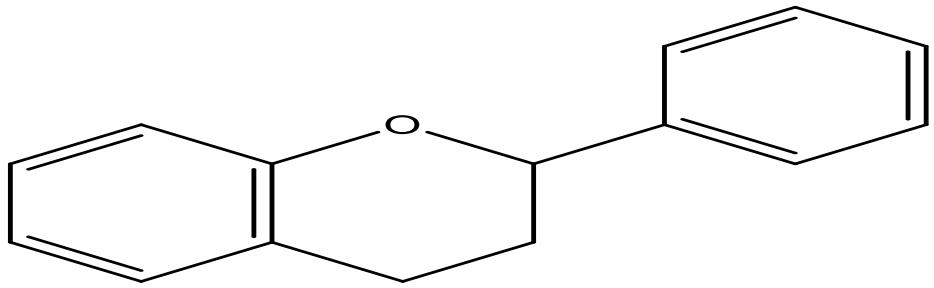


Figure 9: Structure of Flavane (2-phenyl-benzopyrane) basic unit of flavonoids

Flavonoids are groups of polyphenolic plant secondary metabolites. They contribute to the colour of fruits, vegetables and herbs. The abundance of flavonoids coupled with their low toxicity relative to other plant compounds make them to be readily ingested in large quantities by animals, including humans. They are mainly used to treat many important common diseases due to their proven ability to inhibit specific enzymes, simulate some hormones and neurotransmitters and scavenge free radicals. The major classes of flavonoids based on their molecular structures include chalcone, flavone, flavanone, flavonol, anthocyanines and isoflavonoids. Bioactive flavonoids include hesperidin (Figure 10) which is antihypertensive with lipid lowering effects (Chanet *et al.,* 2012) and quercetin (Figure 11) which is antioxidant, antiviral, antibacterial, anti-carcinogenic and anti-inflammatory (Fortunato *et al.,* 2012).

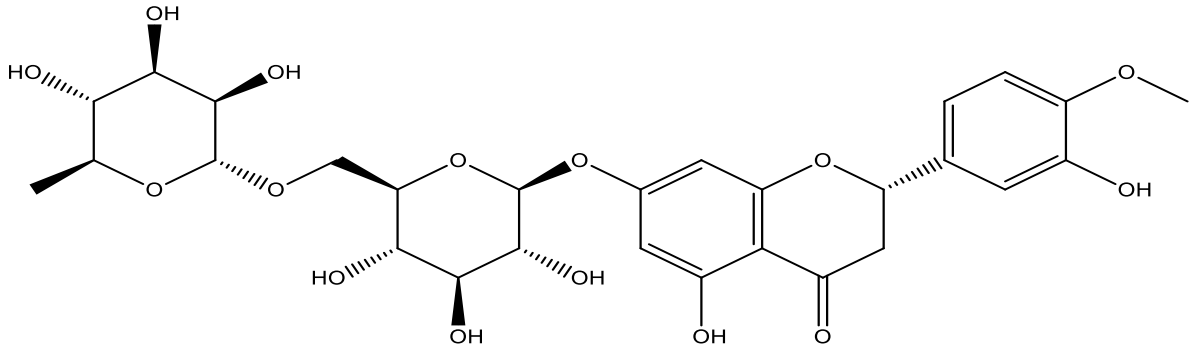


Figure 10: Structure of Hesperidin [(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5- trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one]

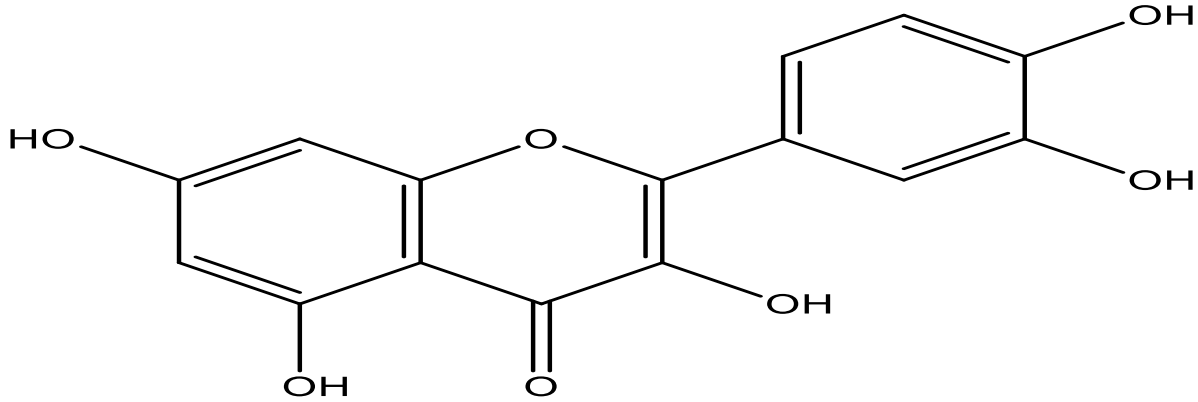


Figure 11: Structure of Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy chromen-4-one)

2.3.4 Glycosides

These are organic natural compounds present in several plants which upon hydrolysis give one or more sugars known as glycones and non-sugars called aglycones. Glycosides convert toxic to non- or less toxic materials, transfer water-insoluble substances by using monosaccharide, serve as sources of energy (sugar reservoir), store harmful products such as phenol and regulate certain functions like growth rate in the systems.

Glycosides can be classified on the basis of the linkage atom between glycone and aglycone moieties into C-, N-, O- and S-glycosides.

In C-Glycosides, the sugar is linked directly to the carbon atom of the aglycone. An example of bioactive C-Glycoside is aloin (Figure 12) which is the main phytoconstituent of aloes and is used as a laxative agent (Patel and Patel, 2013).

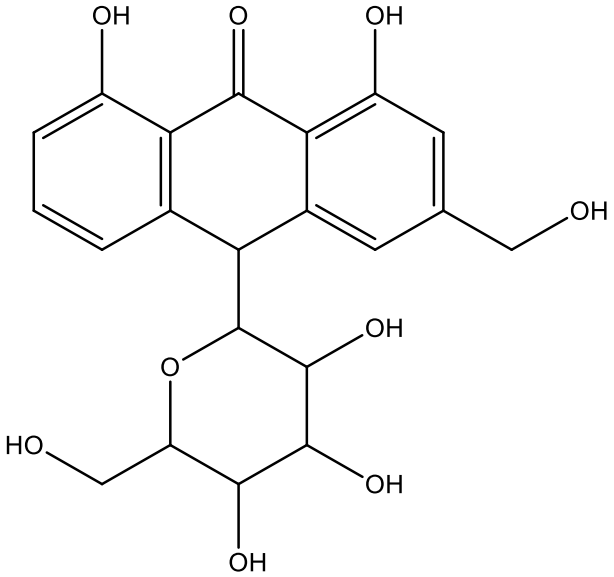


Figure 12: Structure of Aloin (1, 8-dihydroxy-3-(hydroxymethyl)-10-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-10H-anthracen-9-one)

N-Glycoside is generated when a sugar component is linked to an aglycone, through a nitrogen atom, establishing as a result a C-N-C linkage. Adenosine (Figure 13) brings about smooth muscle relaxation which leads to vasodilation (Klabunde, 2011).

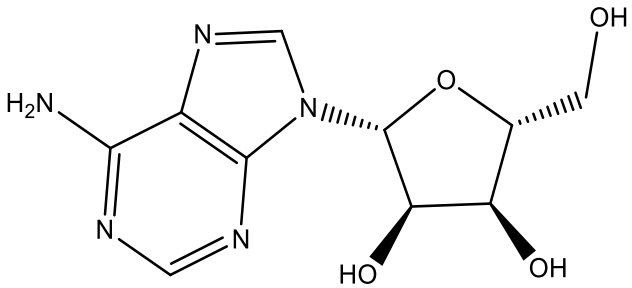


Figure 13: Structure of Adenosine [(2R, 3R, 4S, 5R)-2-(6-aminopurin-9-yl)-5-(hydroxymethyl) oxolane-3,4-diol]

In O-Glycosides, the sugar is bonded to the aglycon through oxygen which can be alcoholic, phenolic or carboxyl group. An example of this type of glycoside is salicin (Figure 14) which is anti-inflammatory. Salicin administration effectively and dose-dependently prevented pro-inflammatory cytokine expression in dextran sulphate sodium (DSS)-induced colitis mice (Verma *et al*., 2014).

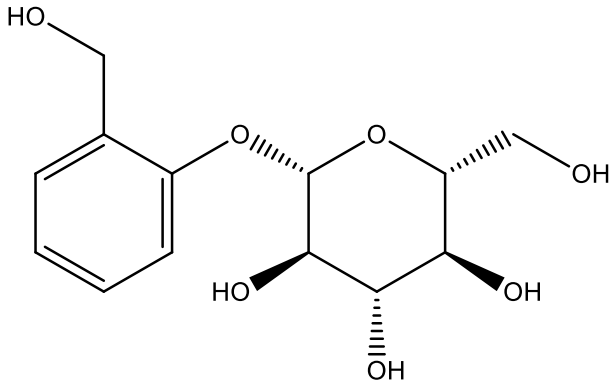


Figure 14: Structure of Salicin [(2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-[2-(hydroxymethyl) phenoxy] oxane-3,4,5-triol]

In S-glycosides, the sugar is attached to a sulfur atom of the aglycone. An example of this is sinigrin (Figure 15). Sinigrin exhibited *in vitro* inhibitory activity against tumors growing in human promyelocytic leukaemia (HL60) cell line (Lozano-Baena *et al*., 2015).

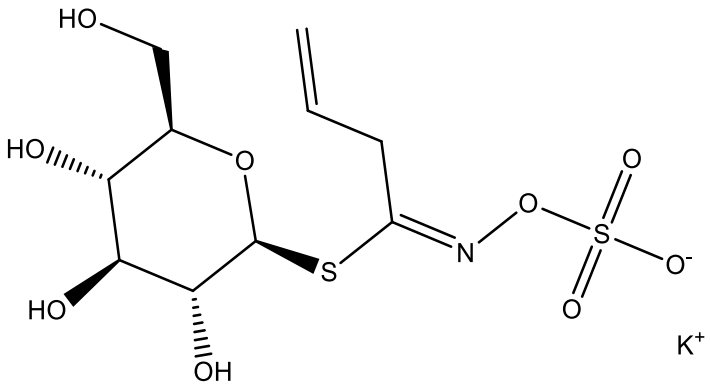


Figure 15: Structure of Sinigrin (Potassium [(E)-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]sulfanylbut-3- enylideneamino] sulfate)

2.3.5 Saponin

Saponins consist of a polycyclic aglycones attached to one or more sugar side chains thus are normally referred to as glucosides. The aglycone, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). Saponins are bitter and have foaming abilities which is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. They are similar to naturally occurring hormones found in the human body. An example of bioactive saponin is gypenoside. Gypenosides (Figure 16), the main components from *Gynostemma pentaphyllum* decreased the percentage of viable human colorectal cancer cells SW-480 established from the primary adenocarcinoma arising in the colon (Yan *et al*., 2014).

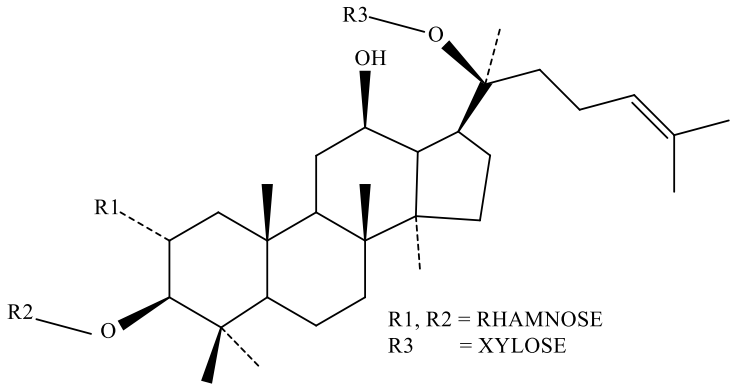


Figure 16: Structure of Gypenoside

2.3.6 Essential Oils

Essential oils, also known as essences, volatile oils, etheric oils, or aetheroleum, are natural products formed by several volatile compounds (Sangwan *et al*., 2001; Hüsnü *et al*, 2007). According to the International Standard Organization on Essential Oils (ISO 9235: 2013) and the European Pharmacopoeia (Council of Europe 2004), an essential oil is defined as the product obtained from plant raw material by hydrodistillation, steam distillation (dry distillation) or by a suitable mechanical process, for citrus fruits (Zuzarte and Salgueiro, 2015). Cold pressing without heat is usually used for citrus fruit oils because their

constituents are thermosensitive and unstable, converting into artifacts under heat and pressure.

The definition of an essential oil excludes other aromatic/volatile products obtained by different extractive techniques like extraction with solvents (concretes, absolutes), supercritical fluid extraction and microwave-assisted extraction. Essential oils also differ from fixed oils or fatty oils in both chemical and physical properties. Fatty oils contain glycerides of fatty acids and leave a permanent stain on filter paper, whereas essential oils contain volatile compounds and vanish rapidly without leaving any stain ((Zuzarte and Salgueiro, 2015).

Essential oils can be found in various plant organs such as flowers, fruits, seeds, leaves, stems, and roots, being produced and stored in secretory structures that differ in morphology, structure, function and distribution.

The composition of essential oils in plants usually varies considerably because of both intrinsic factors such as sexual, seasonal, ontogenetic and genetic variations and extrinsic factors which can be ecological and environmental (Figueiredo *et al*., 2008a; Taiz and Zeiger 2010).

2.3.6.1 Terpenes

Terpenes result from the condensation of a pentacarbonate unit with two unsaturated bonds, known as the isoprene unit (2-methyl-1,3-butadiene), hence, are most times called isoprenoides. The designation “terpenes” was first used by Kekulé in 1880 to name $C_{10}H_{16}$ compounds found in turpentine. In 1887, his assistant Otto Wallace formulated the “isoprene rule” suggesting that terpenes were formed by two or more isoprene units.

Terpenes are classified into different structural and functional classes. According to the number of isoprene units in their structure, terpenes can be classified into hemiterpenes (1 unit), monoterpenes (2 units), sesquiterpenes (3 units), diterpenes (4 units), and so on. The terpenes most often found in essential oils are monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$). These compounds have many isomeric cyclic or linear structures, various degrees of unsaturations, substitutions, and oxygenated derivatives, being generally called terpenoids. Terpenoids are extremely variable, showing different carbon skeletons and a wide variety of oxygenated derivatives, including alcohols, esters, aldehydes, ketones, ethers, peroxydes and phenols.

Some compounds that were found present in essential oils and their plant sources are shown on Table 1.

Table 1: Some constituent compounds of essential oil samples and their plant sources

S/n	Some constituents of essential oil	Classes	Plants	Family	Plant parts	References
1.	Limonene	Terpenes	*Pinus caribaea* Morelet	Pinaceae	Fresh needles	Moronkola *et al.*, 2009
2.	1, 8-Cineole	Ethers	*Eucalyptus maidenii* F. Muell	Myrtaceae	Dried leaves	Sebei *et al.*, 2015
3.	6,10,14-trimethyl-2-pentadecanone	Methylated Ketones	*Euphorbia hirta* L.	Euphorbiaceae	Dried aerial parts	Ogunlesi *et al.*, 2009
4.	n-Hexadecanoic acid, 9,12,15-octadecanoic acid-(Z,Z,Z), dodecanoic acid and tetradecanoic acid	Fatty acids	*Sesamum radiatum* Schumach. & Thonn.	Pedaliaceae	Dried leaves	Ogunlesi *et al.*, 2010
5.	13-heptadelyn-1-ol	Alcohols	*Chansmanthera dependens* Hochst	Menispermaceae	Dried stem	Ogunlesi *et al.*, 2010
6.	Dihydrocarveol acetate	Esters	*Lavandula officinalis* L.	Lamiaceae	Dried aerial parts	Moradkhani *et al.*, 2011
7.	Geranial and neral	Aldehyde	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Dried leaves	Boukhatem *et al.*, 2014
8.	Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol)	Terpenoids	*Calotropis procera* (Aiton) W.T. Aiton	Asclepiadaceae	Dried leaves	Okiei *et al.*, 2009

2.3.6.2 Some Biological Effects of Essential Oils

Some essential oils are good antioxidants. Oxidation damages various biological substances and subsequently causes many diseases, including asthma, cancer, Alzheimer's disease, arthritis, diabetes, Parkinson's disease and atherosclerosis. As a result, many diseases have been treated with antioxidants to prevent oxidative damages (Sepulveda and Watson, 2002). *Thymus spathulifolius* essential oil possess antioxidant activity because of the high content of thymol (36.5%) and carvacrol (29.8%) and the essential oils of *Salvia cryptantha* and *Salvia multicaulis* had been reported to exhibit higher antioxidant activity than those of ascorbic acid and BHT (Sokmen *et al.,* 2004; Tepe *et al.*, 2004).

Essential oils have antibacterial properties against a wide range of bacterial strains, such as *Listeria monocytogenes, Listeria innocua, Salmonella typhimurium, Escherichia coli, Shigella dysenteria, Bacillus cereus, Staphylococcus aureus, S. Typhimurium* (Hulin *et al.,* 1998).

Strong antibacterial effects against *B. cereus, Pseudomonas aeruginosa, and E. coli* were exhibited by mixtures of essential oils which include oregano and thyme, oregano and marjoram, thyme and sage (Gutierrez *et al*., 2008).

Many other bioactivities of essential oils have been reported which include anti-inflammatory and anti-carcinogenic. The fruits essential oil of *Xylopia aethiopica* showed activity against four microorganisms and cytotoxicity to carcinoma cells (Hep-2 cell line) at 5 mg/ml concentration (Asekun and Adeniyi, 2004).

2.4 Asthma and its Causes

Asthma is a highly prevalent disease that involves a complex interplay of inflammation, air flow obstruction and bronchial hyperresponsiveness. It has been established that asthma is not a single disease, but rather a syndrome that can be caused by multiple biologic mechanisms. Thus, asthma encompasses many disease variants with different etiologic and pathophysiologic factors (Ishmael, 2011).

The dominant feature that leads to clinical symptoms of asthma is inflammation and smooth muscle contraction which result in narrowing and obstruction of the airway (Barnes, 2008).

Clinically, asthma can be described based on symptoms that are either intermittent or persistent, and these symptoms are further classified in terms of severity that is, mild, moderate or severe. The many potential triggers of asthma largely explain the different ways in which asthma can present. In most cases, the disease starts in early childhood from 2-6 years of age. In this age group, the cause of asthma is often linked to exposure to allergens, such as dust mites, tobacco smoke, and respiratory infections. In children, less than 2 years of age, asthma can be difficult to diagnose with certainty. Wheezing at this age often follows an infection and might disappear later, without ever progressing to asthma. Asthma, however, can develop again in adulthood. Adult-onset asthma occurs more often in women, mostly middle-aged, and frequently follows a respiratory tract infection. The triggers in this group are usually non-allergic in nature thus, asthma is commonly divided into two types: allergic, also known as extrinsic asthma and non-allergic, otherwise called intrinsic asthma.

Extrinsic, or allergic asthma, is more common and typically the one that develops in childhood. Approximately 70-80% of children with asthma also have documented allergies. Typically, there is a family history of allergies. Additionally, other allergic conditions, such

as nasal allergies or eczema, are often also present. Allergic asthma often goes into remission in early adulthood. However, in many cases, the asthma reappears later.

Intrinsic asthma represents a small amount of all cases. It usually develops after the age of 30 and is not typically associated with allergies. Women are more frequently affected and many cases seem to arise from a respiratory tract infections. The condition can be difficult to treat and symptoms are often chronic and year-round.

2.4.1 Oxidative Stress in Asthma

Oxygen-derived radicals are generated constantly as part of normal aerobic life. They are formed in mitochondria as oxygen is reduced along the electron transport chain. Reactive oxygen species (ROS) are also formed as necessary intermediates in a variety of enzyme reactions. Free radicals in the form of ROS have become increasingly recognized as playing a major role in many disease processes. ROS such as superoxide anion ($O_2^{\cdot -}$) and the hydroxyl radical ($^{\cdot}OH$) are unstable molecules with unpaired electrons, capable of initiating oxidation. This can result in the oxidation of proteins, DNA, and lipids that may cause direct tissue injury or induce a variety of cellular responses, through the generation of secondary metabolic reactive species. The lung exists in a high-oxygen environment and together with its large surface area and blood supply is highly susceptible to injury mediated by oxidative stress. Consequently, the lung contains many antioxidant defences in order to protect itself from oxidant-induced tissue damage. ROS can be generated either endogenously by metabolic reactions or exogenously from cigarette smoke and oxidant gases, such as ozone, nitrogen dioxide and sulphur dioxide, to airborne particulate matters. As a result, increased levels of ROS have been shown to affect the extracellular environment impacting on a variety of physiological processes (Gutteridge & Halliwell, 2000). In addition, ROS can initiate inflammatory responses in the lungs (Rahman and MacNee, 1998). It is proposed that ROS

produced in the body and recruited to sites of inflammation is a major cause of the cell and tissue damage associated with many chronic inflammatory lung diseases including asthma.

Oxidative stress has traditionally been considered to be a toxic byproduct of aerobic metabolism and a factor involved in tissue damage. Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species (ROS) released from inflammatory cells (Kirkham and Rahman, 2006). Oxidative stress aggravates airway inflammation, enhances bronchial hyperresponsiveness, stimulates bronchospasm, and increases mucin secretion thus it plays a critical role in the pathogenesis of bronchial asthma The control of oxidative stress at the appropriate time and with the proper methods is critical for the effective management of asthma.

2.4.2 Inflammation in Asthma

Inflammation is a response of the immune system to injury which is beneficial to the host under normal circumstances. Inflammation also participates importantly in host defences against infectious agents but contributes to the pathophysiology of many chronic diseases which include asthma.

Environmental and inflammatory stimuli induce the production of mediators from the airway epithelium, which activates and recruits inflammatory cells. Inflammatory cells infiltrate the lungs and release mediators that augment the inflammatory response in the epithelium, creating a cycle of chronic inflammation. This process causes bronchoconstriction and epithelial damage, and it can result in the remodeling of the airway.

2.4.3 Infections in Asthma

Infections and asthma are intricately interwoven disease processes. Infections have been associated with asthma on-set, acute exacerbations of asthma and the maintenance of a chronic asthma phenotype. Colonization of the upper airways in infancy with common bacterial pathogens has been demonstrated to increase the risk of subsequent asthma (Guilbert and Denlinger, 2010). Case reports of chronic asthma commencing with *Mycoplasma pneumoniae* infection suggest that this pathogen is a potentially causative agent in some patients.

Mycoplasma pneumoniae and *Chlamydia pneumoniae* are believed to be responsible for acute and chronic forms of asthma in children and adults (Pelaia *et al*., 2006).

It has been reported that neonates colonized in the hypopharyngeal region with *S. pneumoniae, H. influenzae* or *M. catarrhalis*, or with a combination of these organisms, are at increased risk for recurrent, early-life wheezing and asthma at 5 years of age (Bisgaard *et al*., 2007). Another possible mechanism for these associations is that a person with immune function that is biased towards atopy may have both altered host defenses that increase susceptibility to bacterial and viral infections and an increased risk of developing asthma (Kusel *et al.,* 2007). Collectively, these studies regarding chronic bacterial infection have formed the basis for randomized, placebo-controlled, double-blind clinical trials of prolonged courses of macrolide antibiotics on acute and chronic asthma control. The effect of these drugs has been inconclusive as the result has been found to vary with the population (Strunk *et al*., 2008).

2.5 Asthma Management

Many classes of compounds are found useful in the management of asthma. These include antioxidants, anti-inflammatory and antibacterial compounds.

2.5.1 Role of Antioxidants in the Management of Asthma

The harmful effect of free radicals causing potential biological damage is known as oxidative stress (Ridnour *et al*., 2005). Antioxidants offer resistance to oxidative stress by scavenging free radicals and inhibiting cell membrane damage thereby preventing the onset and progression in the management of asthma. Antioxidants represent the physiological first-line of defence against asthma because these molecules exert their action on the cellular signalling processes (Rahman and Adcock, 2006).The lungs have endogenous antioxidant mechanisms to combat the damaging effects of reactive oxygen species (ROS); however, levels of antioxidants in the lungs as well as in blood are reduced in people living with asthma (Grieger *et al.,* 2014). Antioxidant supplements are effective in reducing asthma severity by inhibiting pro-inflammatory agents and neutralizing the effects of excess ROS and reactive nitrogen species (Henricks and Nijkamp, 2001).

An antioxidant, quercetin, caused significant bronchodilation both *in vivo* and *in vitro*. Quercetin proved in laboratory conditions its ability to reduce hyperreactivity of airways which is one of the main attributes of bronchial asthma (Joskova *et al.,* 2011). Quercetin inhibited an enzyme that breaks down signaling proteins which produce swelling, airway narrowing and it caused a relaxation of the airway smooth muscle (Townsend and Emala, 2013). Hence medicinal plants with appreciable quantity of quercetin may have some therapeutic effects on people living with asthma.

2.5.2 Role of Anti-inflammatory Agents in Asthma

Inflammation is a defensive and normal response of the body to any noxious stimulus and is characterised by particular vascular phenomenon (Kajaria *et al.,* 2011). An important medical discovery was that inflammation is present in the bronchial tubes of persons with asthma even when they feel well and when their breathing is normal. The inflammation may be so mild, that it does not cause narrowing of the bronchial tubes. The persistent or chronic presence of the inflammation is responsible for the abnormal narrowing of the bronchial tubes (Christopher *et al*., 2003).

The use of anti-inflammatory medications is a crucial component of the asthma management plan for people who have frequent asthma symptoms. The basic pathology of asthma starts with the process of inflammation. Thus, in investigating the antiasthmatic activity of drugs, a very important step involves demonstrating the anti-inflammatory activity of the drugs (Kajaria *et al.,* 2011).

Anti-inflammatory drugs suppress the inflammatory response in asthma by inhibiting the infiltration and activation of inflammatory cells, the synthesis or release of mediators and the effects of inflammatory mediators themselves (Mali and Dhake, 2011).

2.5.3 Implications of Pathogenic Bacteria in Asthma

Infections and asthma are intricately interwoven disease processes. Infections have been associated with acute exacerbations of asthma, the maintenance of a chronic asthma phenotype, as well as asthma inception (Richard, 2009).

Respiratory tract infections have adverse effects on asthma in terms of disease onset and provocation of exacerbations (Busse and Gern, 2014). *Moraxella catarrhalis* and *Streptococcus pneumoniae* contribute to the severity of respiratory tract illnesses, including

asthma exacerbations (Kloepfer *et al.,* 2014). *Staphylococcus aureus* has been implicated in bacterial infections present in people living with asthma (Tomassen *et al.,* 2013).

Although current treatment such as Inhaled Corticosteroid (ICS) is effective in controlling symptoms, reducing airflow limitations and preventing exacerbations, it does not appear to prevent the underlying severity of asthma. For some patients, the development of chronic inflammation may be associated with permanent alterations in the airway structure referred to as airway remodelling that are not prevented by or fully responsive to currently available treatments (Nele *et al.,* 2001). These problems and numerous side effects of conventional asthma medications had compelled many people living with asthma to resort to the use of medicinal plants.

2.6 Medicinal Plants and Asthma

Medicinal plants have been widely used to treat a variety of infectious and non-infectious diseases in humans. Some plants are used by the practitioners of Alternative Medicine in Nigeria for the management of asthma. Some of these medicinal plants include the plants under study which are*:* *Adansonia digitata* L., *Calotropis procera* (Aiton) W.T. Aiton, *Coix lacryma-jobi* L., *Datura metel* L., *Deinbollia pinnata* (Poir.) Schumach. & Thonn, *Euphorbia hirta* L.and *Pterocarpus osun* Craib.

2.6.1 Botanical and scientific information on *Adansonia digitata* L.

Figure 17: Leaves of *Adansonia digitata* L.

Kingdom Plantae – plantes, Planta, Vegetal, plants

Subkingdom Viridiplantae

Division Tracheophyta – vascular plants, tracheophytes

Subdivision Spermatophytina – spermatophytes, seed plants, phanérogames

Class Magnoliopsida

Order Malvales

Family Malvaceae – mallows, mauves

Genus *Adansonia*

Species *Adansonia digitata* L. – baobab

Adansonia digitata L. which is commonly known as baobab, monkey-bread tree, dead-rat tree or cream-of-tartar tree is a massive deciduous tree which is up to 20–23 m tall. The trunk is often of vast girth with the stout branches near the trunk. The leaves are alternate and simple (in young trees and first leaves of the season in old trees) or digitately compound, at the apex of the branches. The root system extends up to 2 m deep and horizontally further than the height of the tree. *A. digitata* is indigenous to, and widely distributed throughout the savannas and savannah woodlands of sub-Saharan Africa (Wickens and Lowe, 2008).

It is a multi-purpose tree with tender root, tubers, twigs, fruits, seeds, leaves and flowers which are edible. Owing to the nutritional and medicinal benefits of baobab tree parts, it has been used for various purposes in Africa. The leaves are commonly used as vegetables in most African countries and they are eaten both fresh and in dry powdered form. The use of the pulp in food fermentation is a common practice in Nigeria, especially in the northern part of the country where the Fulanis use it in the fermentation of cow milk for 'nono' production.

The phytochemical screening of the ethanolic, methanolic and aqueous leaf extracts of the plant showed the presence of reducing sugar, flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins, phenols and glycosides (Abiona *et al*., 2015).

Adansonia digitata has been alleged to be useful in the management of asthma. The plant, especially the leaves, fruit pulp and the bark is also used for the treatment of malaria, diarrhoea, skin diseases and is known to be demulcent, prophylactic, antihistaminic and antimicrobial (Odugbemi, 2008). The plant which has traditionally been used as an immune stimulant (Kamatou *et al*., 2011), analgesic, antipyretic, febrifuge and astringent are equally used in the treatment of dysentery (Zahra'u *et al*., 2014).

Aqueous extract of *A. digitata* inhibited ethanol–induced gastric ulceration in rats and oral pretreatment with the leaves of the plant caused significant dose–dependent increase both in preventive ratio and percentage ulcer reduction (Karumi *et al*., 2008). The aqueous extract of the fruit pulp exhibited significant antipyretic activities and oral administration of the extract to Balb/c mice at 800 mg/kg had a significant analgesic effect (Ramadan *et al*., 1994). The presence of multiple bioactive compounds in different parts of *A. digitata* has been established and these activities could explain some of the benefits attributed to the traditional leaf and pulp preparations in the treatment of infectious diseases and inflammatory conditions (Vimalanathan and Hudson, 2009).

2.6.2 Botanical and scientific information on *Calotropis procera* (Aiton) W.T. Aiton

Figure 18: Leaves and flowers of *Calotropis procera* (Aiton) W.T. Aiton

Kingdom	Plantae
Subkingdom	Viridaeplantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	*Calotropis*
Species	*Calotropis procera*

Calotropis procera which is commonly known as sodom apple, auricula tree, dead sea apple, desert wick, giant milkweed, king's crown, rooster tree, rubber bush or small crownflower is a shrub or small tree, up to 2.5 m (max. 6 m) in height. The stem is usually simple, rarely

branched, woody at the base and covered with a corky bark while the branches are somewhat succulent (which exude white latex when cut or broken) with simple, opposite leaves.

C. procera has become a serious weed in pastures and overgrazed rangelands. It is native to West, North and East Africa. *C. procera* was formerly placed in the family Asclepiadaceae (the milkweed family), which is now considered a subfamily of the Apocynaceae (Stevens, 2012). The Apocynaceae is a large family of plants including 415 genera and about 4555 species distributed largely throughout the tropics but also in warm temperate climates.

In Nigeria *C. procera* is used by several tribes and the name in the various languages are well documented (Odugbemi, 2008). The plant parts are used as medications for the treatment of asthma and many other diseases which include diarrhoea, dysentery, elephantiasis, leprosy, chronic eczema, ringworm, cough, eye infections and convulsion. The leaves, when crushed and rubbed on the breasts of nursing mothers, increase milk production (Odugbemi, 2008; Ogunlesi *et al*., 2008; Zailani and Ahmed, 2008; Aliero *et al*, 2001). However, the neat latex can cause blindness (Zailani and Ahmed, 2008). Various tribes of Central India use the root, bark and leaves as a curative agent for jaundice (Samvatsar and Diwanji, 2000). Different parts of the plant have been reported to exhibit cytostatic properties (Kumar and Arya, 2006). Phytochemical analysis conducted on the plant parts revealed the presence of saponins, glycosides and simple sugars in the leaves while the root bark was found to contain tannins in addition to those listed (Tahir and Chi, 2002). *C. procera* dried leaf extracts (300 and 600 mg/kg) reduced significantly the level of blood glucose and improved metabolic status of streptozotocin-induced diabetic rats and ameliorate the oral tolerance glucose test (Neto *et al*., 2013). Aqueous fruit and bark extracts of *C. procera* showed antibacterial activities against *Pseudomonas aeruginosa, Salmonella typhi*, *Escherichia coli* and *Streptococcus pyrogenes* at 30 mg/mL (Mainasara *et al*., 2011).

2.6.3 Botanical and scientific information on *Coix lacryma-jobi* L.

Figure 19: Leaves and seeds of *Coix lacryma-jobi* L.

Kingdom Plantae – plantes, Planta, Vegetal, plants

Subkingdom Viridiplantae

Division Tracheophyta – vascular plants, tracheophytes

Subdivision Spermatophytina – spermatophytes, seed plants, phanérogames

Class Magnoliopsida

Order Poales

Family Poaceae – grasses, graminées

Genus *Coix* L. – Job's tears

Species *Coix lacryma-jobi* L. – Job's tears

Coix lacryma-jobi is an annual plant that grows wild to a height of about 3 ft (1 m) in sunny but moist grasslands. The plant has narrow, ribbon-like leaves. *C. lacryma-jobi*is an important crop used as food and herbal medicine in Asian countries (Xi *et al*., 2016) and has now been introduced to almost all tropical and subtropical zones of the world (Lim, 2012). The seed with the husk removed is used medicinally. In some areas the plant is cultivated as a food grain. Although the seed coat is hard to remove, making it difficult to produce flour; *C. lacrymajobi* can be cooked like barley or rice and the flour can be used to make bread. Parched seeds of the plant are used to make tea, and a coffee substitute can be made from the roasted seeds.

The grain and flour of *C. lacryma-jobi* are easily digestible and administered to reduce debility in humans. They are believed to have medicinal values with diuretic, depurative, anti-inflammatory and antitumour activities. A decoction of the leaves is drunk against headache, rheumatism and diabetes. Sap of the stem is applied against insect bites. A decoction of the roots is used as a vermifuge and to treat dysentery, gonorrhoea and menstrual disorders.

Phytochemical investigation of root extracts of *C. lacryma-jobi* revealed the presence of triterpenoids, resins, steroids and fixed oils in petroleum ether extract; flavonoids, triterpenoids, saponins and fixed oils in chloroform extract and alkaloids, carbohydrates, flavonoids, glycosides, resins, saponins, steroids, and tannins in ethanolic extracts (Rajesh *et al.*, 2016).

Hamsters administered with *Bacillus*-fermented *Coix lacryma-jobi* (adlay) experienced significant reduction in serum and hepatic total cholesterol and triglyceride levels (Wang *et al*., 2011). Dietary supplementation with *C. lacryma-jobi* is likely to reduce the risk of

coronary heart disease related to hypercholesterolemia and oxidative stress based on the work of Wang *et al.*, 2012.

2.6.4 Botanical and scientific information on *Datura metel* L.

Figure 20: Leaves and flowers of *Datura metel* L.

Kingdom Plantae – plantes, Planta, Vegetal, plants

Subkingdom Viridiplantae

Division Tracheophyta – vascular plants, tracheophytes

Subdivision Spermatophytina – spermatophytes, seed plants, phanérogames

Class Magnoliopsida

Order Solanales

Family Solanaceae – nightshades, solanacées

Genus *Datura* – jimsonweed, *datura*

Species *Datura metel* L.

Datura metel, commonly known as angel's trumpet, downy thorn apple or metel thorn apple is an annual or short-lived perennial erect herb which grows up to 2 m tall. It is often much-branched with short-hairy stem and alternate, simple leaves. *Datura metel* is native to the Americas and has been introduced from there throughout the tropics and subtropics. It is probably grown throughout tropical Africa as an ornamental plant and is documented as a naturalized weed in many African countries (Nuga and Setshogo, 2008). In tropical Africa as well as in Asia the most widely documented use of *Datura metel* is for relieving asthma, cough, tuberculosis and bronchitis by smoking the dried leaves, roots or flowers as a cigarette or in a pipe (Nuga and Setshogo, 2008). The use of different plant parts in various preparations for sedating patients with mental disorders is widespread. In Africa, other traditional uses of the leaves include the treatment of haemorrhoids, boils, sores, skin diseases, rheumatism, headache, toothache, cholera, parasites such as ringworm and guinea worm, and as an abortifacient and anaesthetic. In Nigeria, *D. metel* is usually abused by adding the decoction of its leaves and fruits to drinks to achieve a "high", as a substitute for marijuana because it is relatively cheap and readily accessible (Adekomi *et al.,* 2010).

The phytochemical test of *Datura metel* showed the presence of mainly alkaloids such as hyoscyamine, hyoscine and atropine (Alexander *et al.,* 2008). A study by Etibor *et al.,* 2015, demonstrated the histological distortion of the medial prefrontal cortex of wistar rats treated with ethanolic seed extracts of *Datura metel* (100 mg/kg and 200 mg/kg body weight, at a dose-dependent rate) for a period of 14 days. The seed powder of *Datura metel* when given to both normal and diabetic rats produced significant reduction in blood glucose at the 8^{th} hour (Murthy *et al*., 2004). However, various parts of the *Datura* plants are toxic though this has been exaggerated through improper dosage, duration of treatment and frequency of administration.

2.6.5 Botanical and scientific information on *Deinbollia pinnata* (Poir.) Schumach. & Thonn

Figure 21: Leaves and fruits of *Deinbollia pinnata* (Poir.) Schumach. & Thonn

Kingdom Plantae - plants

Subkingdom Viridiplantae - green plants

Phylum Tracheophyta - vascular plants

Subphylum Euphyllophytina

Class Spermatopsida core seed plants

Subclass Magnoliidae - angiosperms

Order Sapindales

Family Sapindaceae

Genus *Deinbollia*

Species *Deinbollia pinnata* (Poir.) Schumacher & Thonning

Deinbollia pinnata which is native to Equatorial Guinea and Cameroon is an erect shrub or small tree, 4–15 ft. high normally found in regrowth forest vegetation. It usually has creamy-white flowers with orange fruits.

The roots and the leaves are normally used for cough, bronchial asthma and as an aphrodisiac (Nwauzoma and Dappa, 2013). Traditionally, the seeds of *D. pinnata* and *Capsicum frutescens* with the leaves of *Peperomia pellucida* and *Elytraria marginata* are cooked with fish (*Clarias gariepinus*) and taken as soup in Ijebu Ode, Ogun State, Nigeria for the treatment of measles (Sonibare *et al*., 2009). The phytochemical screening of the n-hexane, ethyl acetate, and methanol extracts of *D. pinnata* revealed the presence of terpenoids, steroids, phenols, flavonoids, saponins and cardiac glycosides (Lasisi *et al.*, 2016).

The methanol extracts from the leaves of *D. pinnata* possess both antioxidant and antimicrobial activities (Sofidiya *et al.*, 2012).

2.6.6 Botanical and scientific information on *Euphorbia hirta* L.

Figure 22: Aerial parts of *Euphorbia hirta* L.

Kingdom Plantae – plantes, Planta, Vegetal, plants

Subkingdom Viridiplantae

Division Tracheophyta – vascular plants, tracheophytes

Subdivision Spermatophytina – spermatophytes, seed plants, phanérogames

Class Magnoliopsida

Order Malpighiales

Family Euphorbiaceae – spurge, euphorbes

Genus *Euphorbia* L. – spurge

Species *Euphorbia hirta* L. – pillpod sandmat

Euphorbia hirta whose common names include asthma herb, snakeweed and pill-bearing spurge is an annual herb with branches up to 50 cm long. All parts of the plant are short hairy with white latex. *E. hirta* is considered a weed with simple opposite leaves and can be a nuisance to crops due to the large number of its seedlings.

E. hirta is native to Central Europe but now commonly occurs throughout the tropics and subtropics including Tropical Africa. *Euphorbia hirta* grows in cultivated fields, gardens, roadsides and waste places, from sea-level up to high altitude.

Euphorbia hirta is an important medicinal herb used throughout its distribution area, including tropical Africa. It is also regarded as an outstanding medication to treat respiratory system disorders including asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs and colds. A decoction of *E. hirta* is useful in asthma and chronic bronchial infections (Rahman and Aktar, 2013). A decoction or infusion of the plant is used to treat gastrointestinal disorders including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting and amoebic dysentery. The leaves are normally mixed with those of *Datura metel* L. in preparing 'asthma cigarettes'. The plant is also used to treat infections of the skin and mucous membranes, including warts, scabies, tinea, thrush, fungal infections, measles, guinea-worm and as an antiseptic to treat wounds, sores and conjunctivitis.

Phytochemical analyses of the crude extracts of *E. hirta* revealed the presence of flavonoids, steroids, alkaloids, tannins and saponins (Kumar *et al*., 2010).

An *in vitro* evaluation of *E. hirta* extracts against the microfilariae (mf) of *Onchocerca volvulus* which transmits onchocerciasis, the second major cause of blindness in the world (Whitcher *et al*., 2001), showed that *E. hirta* immobilised microfilariae at different levels (Attah *et al*., 2013). *E. hirta* may be toxic depending on the mode of administration. The

toxicity of the *E. hirta* extracts on monkey kidney cell (LLCMK2) lines showed that the crude ethanolic extract of *E. hirta* was the least toxic to the LLCMK2 compared to the fractionated forms (Attah *et al*., 2013).

Traditionally, *E. hirta* is also widely used to manage diabetes. The ethanolic extracts (250 and 500 mg/kg) of leaves, flowers and stem of the plant were evaluated for antidiabetic activity against normal and streptozotocin (STZ)-induced diabetic mice. Oral administration of the extracts for 21 days resulted in a significant reduction in blood glucose level. Chronic effects of the extracts on serum biochemistry were also studied and it was found that serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels were decreased significantly by all the extracts but HDL levels and total proteins were found to be increased after treatments (Kumar *et al*., 2010).

2.6.7 Botanical and scientific information on *Pterocarpus osun* Craib

Figure 23: Leaves of *Pterocarpus osun* Craib

Kingdom Plantae – plantes, Planta, Vegetal, plants

Subkingdom Viridiplantae

Division Tracheophyta – vascular plants, tracheophytes

Subdivision Spermatophytina – spermatophytes, seed plants, phanérogames

Class Magnoliopsida - Dicotyledon

Order Fabales

Family Fabaceae – peas, legumes

Genus *Pterocarpus* Jacq.

Species *Pterocarpus osun* Craib

Pterocarpus osun is commonly known as African rose wood or camwood. It is an evergreen or deciduous small to medium-sized tree up to 30 m tall though it could usually be much smaller. *P. osun* contains fairly hard heartwood with reddish sap from the bark which is normally rubbed on infants for skin protection and used for dyeing especially in traditional sculptures. *Pterocarpus osun* is endemic to southern Nigeria, Cameroon and Equatorial Guinea.

Locally, the stem is an ingredient of traditional medicines against sickle-cell disorder and amenorrhoea. The powdered stem is applied topically to treat skin diseases, to prevent infections of the freshly severed umbilical cord, to treat stiff joints, sprains, rheumatic complaints, and to promote healing of fractured bones. *P. osun* has been found useful in the management of asthma in Alternative Medicine (Odugbemi, 2008).

The crude ethanolic extracts of *P. osun* showed the presence of phenol, saponins, tannins, cardiac glycosides, sterols and terpenes. The elemental analysis revealed the presence of Calcium (Ca), Chromium (Cr), Copper (Cu), Magnesium (Mg), Manganese (Mn), Nickel (Ni), Zinc (Zn), Sodium (Na) and Potassium (K) for all leaf extracts (Abayomi *et al*., 2015).

P. osun leaves attenuated acetaminophen-induced redox imbalance, possibly acting as free radical scavenger, inducer of antioxidant and drug-detoxifying enzymes, which reduced lipid peroxidation (Ajiboye *et al*., 2010).

Table 2: Summary of the ethnobotanical uses of the Medicinal plants under study

(Odugbemi, 2006)

S/N	Botanical Names	Family Names	Common Names	Local Names (Yoruba)	Parts used	Diseases
1.	*Adansonia digitata* L.	Malvaceae	Baobab tree	Ose	Leaves, fruit pulp, bark	Malaria, asthma, diarrhoea, kidney and bladder diseases, demulcent, prophylactics, skin diseases, antimicrobial
2.	*Calotropis procera* (Aiton) W.T. Aiton	Asclepiadeceae	Giant milk weed	Bomu bomu	Leaves, root, bark, latex	Diarrhoea, dysentery, elephantiasis, leprosy, chronic eczema, ringworm, cough, asthma, convulsion, antipyretic
3.	*Coix lacryma-jobi* L.	Poaceae	Job's tear	Aka-ila	Leaves, roots fruits,	Diuretic, antihelmentics, asthma, stimulant, conjuctivities, irregular menstruation.
4.	*Datura metel* L.	Solanaceae	Hairy thorn apple	Apikan	Leaves	Asthma, veneral diseases, convulsion
5.	*Deinbollia pinnata* (Poir.) Schumach. & Thonn.	Sapindaceae	Water willow	Ogiri-egba	Leaves, root	Cough, asthma, aphrodisiac
6.	*Euphorbia hirta* L.	Euphorbiaceae	Asthma weed	Emi-ile	Aerial parts	Cough, asthma, catarrh, hay fever, pulmonary ailment, amoebic dysentery, conjunctivitis, aphrodisiac, hypertension, antispasmodic
7.	*Pterocarpus osun* Craib	Fabaceae	Imo-osun	African rose wood	Root, stem bark	Asthma, fungal and bacterial skin infections, eczema, acne, candidiasis, antipyretic

2.7 Preparation of Plant Materials

Sample preparation is the crucial first step in the analysis of medicinal plants. The basic processes involved in sample preparation include: selection, collection, identification, drying, grinding and extraction.

2.7.1 Selection

A targeted approach towards plant selection is often preferred to a random technique. The plant material to be investigated can be selected on the basis of some specific traditional ethnomedical uses. Extracts prepared from plants and used as traditional remedies to treat certain diseases are more likely to contain biologically active components of medicinal interest. Alternatively, the plant can be selected based on chemotaxonomical data. This means that if species/genera related to the plant under investigation are known to contain specific compounds, then the plant itself can be expected to contain similar compounds. Another approach is to select the plant with a view to investigate a specific pharmacological activity. Additionally, work can be carried out on a particular plant family, or on plants from a specific country or local area and plants can equally be selected following a combination of approaches. The use of literature databases early in the selection process can provide some preliminary information on the type of compounds already isolated from the plant and the methods employed.

2.7.2 Collection and Identification

The whole plant or a particular plant part can be collected depending on where the metabolites of interest (if they are known) accumulate. Aerial (leaves, stems, flowers, fruits, seeds, bark) and underground (bulbs, tubers, roots) parts of a plant can be collected separately. Only healthy specimens should be obtained, as signs of contamination (fungal, bacterial or viral) may be linked to a change in the profile of metabolites present. Collection

of plant material can also be influenced by other factors such as the age of the plant and environmental conditions (e.g., temperature, rainfall, amount of daylight, soil and altitude). It is important to take these issues into account for recollection purposes to ensure a reproducible profile (nature and amount) of metabolites. The plant must also be identified correctly in which case, a botanist or specialized taxonomist should be involved in the detailed authentication of the plant (that is, classification into its species, genus, family, order and class). Features relating to the collection, such as the name of the plant, the identity of the part(s) collected, the place and date of collection, should be recorded as part of a voucher (a dried specimen pressed between sheets of paper) deposited in a herbarium for future reference.

2.7.3 Drying and Pulverization

The plants which are known to contain volatile or thermolabile compounds, may be subjected to snap–freezing as soon as possible after collection. In the laboratory, the collected plants are washed or gently brushed to remove soil and other debris. Frozen samples can be stored in a freezer (at about 20^0C) or freeze-dried. Shredded wet plants are usually extracted immediately or stored in a freezer to prevent any change in the profile of the metabolites. It is, however, a more common practice to leave the sample to dry on trays at ambient temperature and in a dust-free room with adequate ventilation. Environments that are not humid are essential for drying to prevent microbial fermentation and subsequent degradation of the metabolites. Plant materials should be shredded and spread evenly on clean surfaces to facilitate homogenous drying. Protection from direct sunlight is necessary to minimize chemical reactions such as oxidation which can be induced by ultraviolet rays.The plant materials can be dried in an oven in order to accelerate the drying process. This can also minimize enzymatic reactions for example, hydrolysis of glycosides that can occur in the presence of residual moisture in the plant materials. Mechanical grinders are employed

conveniently to pulverize the dried plant materials into various particle sizes. Potential problems of pulverization include the fact that some materials such as seeds and fruits which are rich in fats and volatile oils may clog up the sieves and the heat generated may degrade thermolabile metabolites. The pulverized samples should be stored in sealed containers and kept in a dry and cool place. However, storage for prolonged periods should be avoided, as some constituents may be affected. Pulverization of the plants facilitate the subsequent extraction process by rendering them more homogenous, increasing their surface area and accelerating the penetration of solvent into the cells.

2.7.4 Extraction

Extraction of plants is the separation of medicinally active portions of plants using selective solvents through standard procedures (Handa *et al.,* 2008). Extraction is the crucial first step in the analysis of medicinal plants, and thereafter the extracts are fractionated and characterized. The purpose of extraction is to separate the soluble, relatively complex mixtures of plant metabolites which can be in liquid, semisolid or in dry powdered form from the residue [Azwanida, 2015].

Several solvents can be employed in the extraction of plant materials. Water is used as an extractant in many traditional protocols. Organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various rates of solubility of plant constituents in these solvents. Some techniques involved in plant extraction include: hydrodistillation, partitioning, soxhlet extraction, accelerated solvent extraction (ASE), solid-phase extraction (SPE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE).

2.7.4.1 Hydrodistillation

Hydrodistillation is usually carried out using a Clevenger apparatus and is employed in the extraction of essential oils from plants. In hydrodistillation, the plant materials are normally placed in a round-bottomed flask containing sufficient amount of water and connected to a condenser. The flask is heated by a heating mantle and the essential oil is released from the oil glands in the plant tissue by hot water and steam. Membranes of plant cells are almost impermeable to volatile oils. In the actual process, at the temperature of boiling water, a part of the volatile oil dissolves in the water present within the glands, and this oil-water solution permeates the swollen membranes and finally reaches the outer surface, where the oil is vaporized by passing steam. The vapour mixture of water and oil is condensed by cooling water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water. This distillate flows into an arm of the Clevenger which contains water and hexane. The essential oil is dissolved in the upper hexane layer and is collected. Hydrodistillation method is very simple. However, prolonged action of hot water can cause hydrolysis of some constituents of the essential oils such as esters which reacts with the water at high temperatures to form acids and alcohols.

Hydrodistillation has been adopted by many scholars in numerous research procedures. Extraction of essential oils by hydrodistillation has been reported extensively in scientific literatures. For examples, the extraction of essential oils from the dried leaves of *Euphorbia hirta* Linn. (Ogunlesi *et al*., 2009) and in the GC-MS analyses of the chemical constituents of the leaf, stem, root and seed essential oils of *Aframomum melegueta* (K. Schum) from South West Nigeria (Owokotomo *et al.,* 2014).

2.7.4.2 Soxhlet Extraction

Soxhlet extraction, is a convenient method for the extraction of small to moderate quantities of plant materials. A sample of the plant in a cellulose thimble is placed in a soxhlet extractor which is fitted to a round-bottomed flask. The solvent is placed in the flask and heated in a heating mantle. Typical solvents include methanol, butanol, ethyl acetate, dichloromethane and hexane. The extraction takes place in a closed system whereby the solvent is continually recycled and fresh solvents are used for the extraction. The amount of solvent required for the extraction is small compared to when the plant material is mixed with the solvent. The extraction is usually carried out in cycles or in hours which can be up to 72 hours depending most of the time, on when the colour of the extract becomes clear. The mixture of extract and solvent is filtered and the filtrate evaporated in a rotary evaporator to obtain the concentrated extract. Though the heat needed to drive the extraction will likely cause thermolabile constituents to form decomposition products, soxhlet procedure remains a widely used exhaustive extraction technique (Anderson and Luthria, 2004). Soxhlet extraction was used in the extraction of numerous phytochemicals from the leaf powder of *Azadirachta indica* (Hossain *et al.*, 2013).

2.7.4.3 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is a solid-liquid extraction process performed at elevated temperatures, usually between 200 and 500^0C, and at pressures between 10 and 15 MPa. Samples are packed with inert materials such as sand in the stainless steel extraction cell to prevent them from aggregating and blocking the system tubing. In the procedure, the solid samples are loaded into sample cells of volume 1 - 100 mL and the end caps are tightened onto the cells. The sample cells are thereafter placed on the cell tray and collection bottles loaded onto a collection tray. A robotic arm transfers each cell into the oven which is maintained at the operating temperature. The cell design and associated fluid apparatus allow

operation of the extractions at elevated pressures to maintain the solvents as liquids at temperatures above their boiling points. Once the cell is placed in the oven, the pump immediately begins to deliver the solvent of choice to the sample cell. High pressure allows the extraction cell to be filled faster and helps to force liquid into the solid matrix. This automated extraction technology controls temperature and pressure for each sample and requires less than an hour for an extraction. High temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state while safe and rapid extraction process is usually achieved. ASE requires a drastic reduction in the amount of solvent and extraction time when compared to the traditional soxhlet extraction. However, ASE when performed at high temperature may lead to degradation of thermolabile compounds. High recoveries (~94%) of flavonoids from *Rheum palmatun* were observed using 80% aqueous methanol by ASE (Tan *et al*., 2014).

2.7.4.4 Solid Phase Extraction

Solid-phase extraction (SPE) is a sample preparation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture based on differences in their physical and chemical properties. SPE is normally used to concentrate and purify samples for analysis or to isolate analytes of interest from a wide variety of matrices (Augusto *et al.,* 2013). It can also be used to clean up a sample before using a chromatographic or other analytical methods. The differences in the affinity of the constituents in the mobile phase for the stationary phase is the basis of separation of mixtures by SPE. In SPE technique, the types of sorbent holders commonly used include free disks, which are generally 47 mm in diameter, disks in syringe barrels or cartridges, which vary in size from microsized disks in 1 - 6 mL syringe and a 96-well microtiter plate that uses the 1mL disk. Three different types of SPE exist and they include: normal phase SPE which involves the use of non polar mobile phase and polar stationary phase, reversed phase SPE

which uses polar mobile phase and non polar stationary phase and ion exchange SPE whereby buffer is used as the mobile phase and anionic or cationic exchanger as stationary phase. The primary extraction properties of common sorbent types are shown in Table 3.

Table 3: Sorbent types and extraction properties of common SPE sorbents

Bonded Phase	Primary Properties
Octadecyl (C_{18})	Non-polar
Octyl (C_8)	Non-polar
Ethyl	Non-polar
Phenyl	Non-polar
Cyclohexyl	Non-polar
Cyanopropyl	Non-polar/polar
Propanediol	Polar/non-polar
Silica (unbonded)	Polar
Alumina (unbonded)	Polar
Florisil (unbonded)	Polar
Diethylaminoethyl	Weak anion exchange/polar
Aminopropyl	Weak anion exchange/polar
Carboxyethyl	Weak cation exchange
Propylsulfonic acid	Strong cation exchange
Ethylbenzene sulfonic acid	Strong cation exchange
Trimethylammonium propyl	Strong anion exchange

Reversed-phase procedure has become the predominant mode of SPE whereby a gradient of strongly polar solvent to weakly polar solvents [from weak to strong solvent elution strength] with a non-polar packing materials are normally used and the procedure is as follows:

a. Solvation of the silica-bonded phase or polymer packing with six to ten hold-up volumes of methanol or acetonitrile. The cartridge is then flushed with six to ten hold-up volumes of water or buffer. The cartridge must never be allowed to dry out.
b. Application of the sample which is dissolved in a strongly polar solvent, typically water, on the stationary phase.
c. Elution of the weakly bound components with strongly polar solvents, fairly retained ones with medium polar solvents while more tightly bound components are then eluted with progressively non-polar solvents with increasing elution strength.
d. Evaporation of the solvents to obtain a concentrated extracts which will be used for analyses.

The used cartridge must be discarded after the extraction in a safe and appropriate manner.

Solid phase extraction is regarded as an alternative to liquid-liquid extraction because of its simplicity, low cost and easy automation. Tsiaganis *et al*., 2006 reported the use of solid phase extraction in the separation of total lipids isolated from *Allium cepa, Allium sativum* and *Allium porrum* into neurolipids, glycolipids and phospholipids.

2.7.4.5 Microwave Assisted Extraction (MAE)

Microwaves are non-ionizing waves positioned between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz (Tatke and Jaiswal, 2011). Microwaves heat up molecules by dual mechanism of ionic conduction and dipole rotation (Tatke and Jaiswal, 2011). The microwave extraction assembly comprises mainly of a magnetron which is responsible for the generation of microwaves, a wave guide for directing the propagation of microwave from the source to the microwave cavity, the

applicator, where the sample holder along with the sample is placed and the circulator which regulates the movement of microwaves.

Microwave systems for extraction are available either in closed vessels or focused microwave ovens. Extraction with a closed vessel system allows random dispersion of microwave radiation within the microwave cavity, so that every zone in the cavity and sample is irradiated evenly whereas in focused microwave assisted extraction, only the part of the extraction vessel containing the sample is focused for irradiation with microwave. The targets for heating in dried plant materials are the minute microscopic traces of moisture that occur in plant cells and on heating up this moisture, there is generation of tremendous pressure which brings about the rupture of the cell wall and subsequent exudation of the constituents. The technique requires less solvent volume, gives high and fast extraction performance and offers protection to thermo-labile constituents. However, in non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only (Handa *et al.,* 2008). MAE can be considered as selective method that favour polar molecules and solvents with high dielectric constant. MAE was adopted in the isolation of mangiferin, a glycoside, from the dried leaves of *Mangifera indica* (Salomon *et al.,* 2014).

2.7.4.6 Supercritical Fluid Extraction (SFE)

Supercritical fluid (SCF) also known as dense-gas is a substance that shares the physical properties of both gas and liquid at its critical point which is the highest temperature and pressure at which the substance can exist in vapor–liquid equilibrium (Azwanida, 2015, Nahar and Sarker, 2012). At temperatures and pressures above this point, a single homogeneous fluid, known as supercritical fluid, is formed. Supercritical fluids are increasingly replacing organic solvents, such as n-hexane, dichloromethane and chloroform, which are conventionally used in industrial extractions, purification and recrystallization

operations because of regulatory and environmental laws on hydrocarbons and ozone-depleting emissions. SCFs can have solvating powers similar to organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension. The most commonly used SCF is supercritical CO_2. Other SCFs incude ethane, butane, pentane, nitrous oxide, ammonia, trifluoromethane and water. The solvating power of an SCF can be adjusted by changing the pressure or temperature, or adding modifiers such as 1-10% MeOH which increase the polarity of supercritical CO_2.

Supercritical fluid extraction is the process of separating components from the matrix using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix such as plant material, but it can also be from liquids. A typical SFE system includes a CO_2 source, a pump to pressurize the gas, an oven containing the extraction vessel, a restrictor to maintain high pressure in the extraction line, and a trapping vessel or analyte-collection device. SFE can be used for extracting essential oils from plants, or to strip unwanted materials from a product, for example, decaffeination.

The SFE technique combines the liquid-like solvating capabilities and gas-like transport properties of supercritical fluids for efficient extraction. In natural product extraction and isolation, supercritical fluid extraction (SFE), especially that employing supercritical CO_2, has become the method of choice (Nahar and Sarker, 2012).

Fully integrated and automated SFE systems of various sizes are currently available. Supercritical CO_2 is widely used as an extraction solvent because it is a chemically inert, has low toxicity and is easily removed from the extract. It has a relatively low critical temperature of 304.1K and pressure of 7.38 MPa, thus, thermal decomposition of sample is reduced (Lang and Wai, 2001). The solubility of polar substances in nonpolar CO_2 is quite low; however, if a small amount of a polar modifier (cosolvent) is added, the solubility of polar solutes can be

significantly increased (Lang and Wai, 2001). SFE extraction of flavonoids from orange peels (*Citrus sinensis*) was reported by Toledo-Guillen *et al.,* 2010 and the use of ethanol as a cosolvent in the the decaffeination of green tea by supercritical fluid extraction was reported by Sun *et al*., 2010.

2.8 Plant Analyses

2.8.1 Chromatography

Chromatography is a separation technique whereby the components of a mixture are distributed between two phases, the stationary phase and the mobile phase. The mobile phase moves through or over the surface of the stationary phase. The components of the mixture have different affinities for each phase, hence some are retained longer on the stationary phase than others causing separation. The retention of a component is determined by the chemical and physical properties of the two phases and the experimental conditions especially the temperature and pressure.

The term 'liquid chromatography' covers a range of chromatographic systems, including liquid-solid, liquid-liquid, ion exchange and size exclusion chromatography, all of which employ a mobile liquid phase.

Pure reference materials must be chromatographed under the same conditions as the test samples and the identity of the compounds must be confirmed by other analytical method/s.

The most common chromatographic methods include: thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC or simply GC).

Table 4: Classification of Chromatographic Methods

	Stationary Phase	**Mobile Phase**	**Mechanism**	**Principal Application**
Planar Chromatography				
Paper Chromatography	Paper (cellulose)	Liquid	Adsorption	Novel tripeptide from *Azadirachta indica (*Rajeswari *et al*., 2013)
Thin Layer Chromatography (TLC)	Silica, cellulose	Liquid	Adsorption	Berberine and palmatine alkaloids from stem bark of *Mahonia manipunensis* (Pfoze *et al*., 2014)
Liquid Chromatography				
High Performance Liquid Chromatography (HPLC)	Liquid on solid support	Liquid	Partition	Saponin from *Moringa oleifera* pods (Sharma *et al.,* 2013)
Ion Exchange Chromatography (IEC)	Ion exchange resin	Liquid	Ion exchange	Lignan compounds from flax seed (Al-Jumaily *et al*., 2012)
Size Exclusion Chromatography (SEC)	Silica, polymeric gel	Liquid	Exclusion	Proteins from flaxseed (Tehrani *etal.,* 2014)
Gas Chromatography				
Gas Liquid Chromatography (GLC or GC)	Methyl siloxane as inner coating of the column	Gas	Partition	Essential oil from *Sesamun radiatum* (Ogunlesi *et al*., 2010)

2.8.2 Thin-Layer Chromatography (TLC)

A thin-layer chromatography system consists of a finely divided particulate stationary phase immobilised as a thin layer on a solid support and a liquid mobile phase consisting of an organic solvent or mixture of solvents. The stationary phase can be aluminium oxide, magnesium oxide, silica oxide, cellulose, ion exchange resins and chiral selectors coated to a depth of 0.15 to 0.5 mm on glass, aluminium or plastic sheets (varying in size from 20 cm by 20 cm plates to microscope slides). The mobile phase can be single solvent or mixtures of

two or more solvents ranging from non-polar (hydrocarbons) to polar solvents (alcohols, water, acids and bases) having the appropriate polarity to get the required separation. The ideal solvent-system is simply the one that gives the best separation.

TLC is normally performed under normal temperature and atmospheric pressure. The liquid sample or dissolved sample in a volatile solvent, is deposited as a spot on the stationary phase. The mixture is separated as it moves through a thin layer of stationary phase by the migrating solvent (mobile phase). The sample ascends the plate by capillary action of the mobile phase and the various components of the sample are retarded in proportion to their interaction with (sorption on) the sorbent bed. A separation is effected as the various components travel up the plate and the result of TLC can be expressed using the retardation factor (R_f) value.

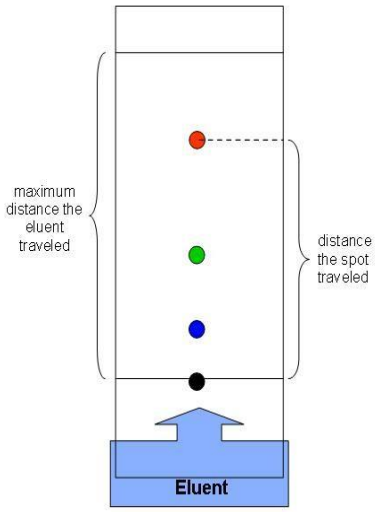


Figure 24: TLC plates showing different spots during separation

$$R_f value = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent\ front}$$

Equation 1: Calculation of R_f value

The optimum separation of compounds by TLC is usually achieved when R_f values are between 0.3–0.5. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. The polarity of the compound determines the relative positions of the components upon the TLC plate. Reversed phase TLC using a polar eluent on a non-polar stationary phase is a useful method to try out separations that could then be carried out by reversed-phase HPLC.

2.8.3 Gas Chromatography (GC)

Gas chromatography is a separation technique in which the components of a sample partition between the stationary and mobile phase. As the name indicates the system operates with the flow of gas in the procedure and was especially designed to evaluate volatile compounds which include essential oils and fatty acids. The gas is set to flow at a constant rate from the cylinder on to the liquid layer impregnated on solid support in a column. The sample is injected into the injection point and is carried by the mobile gas into the column. Inside the column, the components get separated by differential partition between the mobile gas phase and stationary liquid phase. The component that partitioned into gas comes out of the column first and is detected by detector. The ones partitioned into liquid phase comes out later and is also detected. The recordings are displayed onto a computer usually as peaks. From these peaks one can identify the components and also their concentrations.

2.8.3.1 Instrument overview

The schematic diagram of a typical GC is shown in Figure 25.

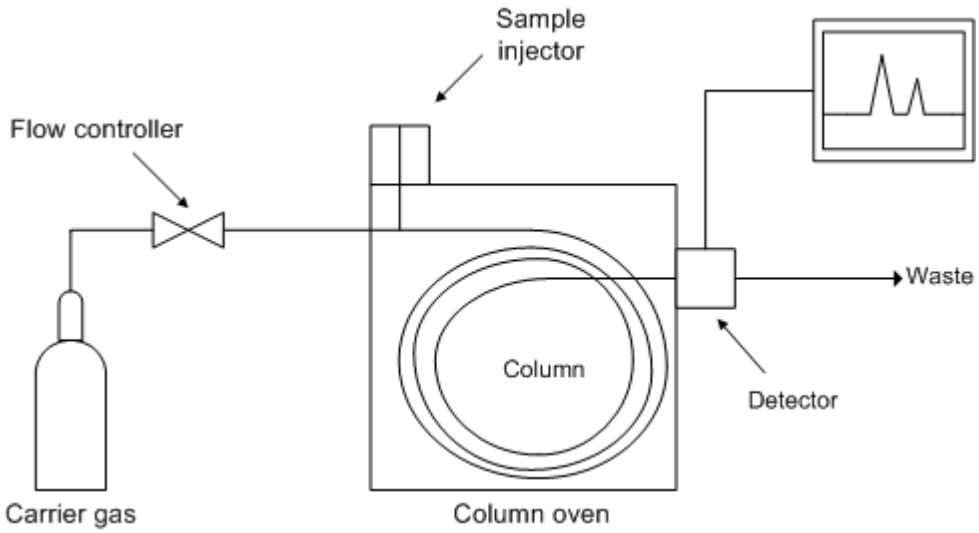


Figure 25: The schematic diagram of a Gas Chromatograph

The basic components of a gas chromatograph include the carrier gas, injector, column and the detector.

2.8.3.2 The Carrier Gas

The carrier gas, known generally as mobile phase in GC, carries the sample molecules along the column while it is not dissolved in or adsorbed on the stationary phase. The carrier gas is inert and does not interact with the sample, thus, GC separation's selectivity can be attributed to the stationary phase alone. However, the choice of carrier gas is important to maintain high efficiency. High purity hydrogen, helium, nitrogen and argon are commonly used for gas chromatography and choice depends on factors such as availability, purity and the type of detector employed. For example, helium is preferred when thermal conductivity detectors are employed because of its high thermal conductivity relative to the vapour of most organic compounds. Gas chromatographs can be operated isothermally which means one selected temperature is used throughout a separation or the temperature can also be programmed.

2.8.3.3 The injector

This is the place where the sample is volatilized and quantitatively introduced into the carrier gas stream. Usually a syringe is used for injecting the sample into the injection port which can be done manually or with autosamplers. The most common methods of injection include split/splitless injection, programmed temperature vaporiser, and cold on-column injection. The choice of methods depends on the concentration range of the target analytes and their physico-chemical properties.

A split injection mode involves the delivering of small volume of sample extract (typically in a range of 0.1–2 μL) rapidly into a glass lined heated injection port, where it vaporises and mixes with carrier gas; a preset proportion, normally between 1 – 10% is allowed to pass into the column and the rest is vented to the atmosphere. This avoids problems with overloading, but the technique only works if the concentrations of materials in the sample are high (greater than 0.01%). However, considering the loss of most of the injected samples which depends on the setting of split ratio, this technique is obviously not suitable for trace analysis where very low detection limits are required.

Splitless injection is often the most appropriate for samples with low concentration of materials. In this mode all the analyte samples vaporized in the injector goes onto the column This method involves the injection of a larger volume, between 0.5 – 5 μL of a dilute solution of the sample in a volatile solvent but with the split vent closed and the column temperature set at about 20 – 25 ^{0}C lower than the boiling point of the solvent. The ideal splitless hold time is long enough to allow most of the vaporized samples in the injection port liner to be transferred to the analytical column. Very long splitless hold times can produce tailing and broad peaks.

2.8.3.4 The GC Column

The gas chromatographic column may be considered as the heart of the GC system where the separation of sample components takes place. GC columns are classified as either packed or capillary columns.

Packed column is typically a glass or stainless steel coil that is filled with the stationary phase, or a packing coated with the stationary phase. Packed columns contain a finely divided, inert, solid support materials (commonly based on diatomaceous earth) coated with liquid stationary phase. Capillary column is a thin fused-silica, that is, purified silicate glass capillary that has the stationary phase coated on the inner surface. Capillary columns provide much higher separation efficiency than packed columns but are more easily overloaded by many samples. The comparison of packed and capillary column characteristics are shown on Table 5.

Table 5: Comparison of packed and capillary column characteristics

	Packed column	**Capillary column**
Outer diameter	2 m	30 m
Inner diameter	2 mm	250 µm
Liquid phase loading / Film thickness	5 % wt/wt	0.25 µm
Total mass of stationary phase (mg)	160	6
Individual solute capacity	>8000 ng	20 ng
Outlet flowrate (mL/min)	15	1
Holdup time	15 sec	120 sec
Plates/meter	2500	3500
Total plates	5000	105,000
Resolution for peak	2.6	12
Cost	-	About 4 times more expensive

The stationary phase is contained in the column and GC can be classified into gas-solid chromatography (GSC) and gas-liquid chromatography (GLC) according to the nature of the stationary phase. In GSC, the stationary phase comprises of active solid adsorbents which

conventionally are porous polymers or materials such as activated carbon, molecular sieve, silica and alumina powder packed in columns. The active solid supports provide adsorbent surfaces on which selective adsorption and desorption of the volatile components take place.

GLC is more widely used than GSC and is often synonymously referred to as Gas chromatography (GC). In this technique a non-volatile liquid (usually high boiling polymer) is coated as a thin layer on an inactive solid support or on the inside wall of the capillary tubing. The sample components partition between the liquid film and the carrier gas. The inert support serves to increase the surface area of the liquid film for greater interaction with sample components. The separation of compounds is based on the different strengths of interaction of the compounds with the stationary phase ("like-dissolves-like" rule). The stronger the interaction between a compound and the stationary phase, the longer the retention time. If the polarity of the stationary phase and a compound is similar, the retention time increases because the compound interacts stronger with the stationary phase. As a result, polar compounds have long retention times on polar stationary phases and shorter retention times on non-polar ones using the same temperature.

2.8.3.5 The GC Detectors

GC detectors detect the isolated components and help in identification and quantification of the samples. The detectors in GC can be flame ionisation detector, thermal conductivity detector, electron capture detector and mass spectrometer.

2.8.3.5.1 Flame Ionization Detector

A flame ionization detector (FID) is a frequently used gas chromatographic detector that measures the concentration of organic species in a gas stream. The operation of the FID is based on the detection of ions formed during combustion of the organic compounds in a hydrogen flame.

The sample is introduced into a hydrogen flame inside the FID and hydrocarbons in the sample will produce ions when they are burnt. To detect these ions, two electrodes are used to provide a potential difference. The positive electrode doubles as the nozzle head where the flame is produced while the other, negative electrode is positioned above the flame as a collector plate. The ions thus are attracted to the collector plate and upon hitting the plate, induce a current. The current across this collector is thus proportional to the rate of ionisation which in turn depends upon the concentration of hydrocarbon in the sample gas. The more carbon atoms in the molecule, the more fragments are formed and the more sensitive the detector is for this compound.

This current is measured and fed into an integrator. The manner in which the final data is displayed is based on the computer and software. In general, a graph is displayed that has time on the x-axis and total ion on the y-axis. FID are used widely in GC because they have low acquisition and maintenance cost, ruggedly constructed and can measure very low and high concentrations. A typical slow analyser might have a response time of 1-2 seconds.

However, FID cannot detect inorganic and oxygenated compounds or those with functional groups and they are not good for preparatory GC.

2.8.3.5.2 Thermal Conductivity Detector (TCD)

This detector is less sensitive than the FID, but it is well suited for preparative applications, because the samples are usually not destroyed. The detection is based on the comparison of two gas streams, one containing only the carrier gas with the one with the carrier gas and the compound. Naturally, a carrier gas with a high thermal conductivity such as helium or hydrogen is used in order to maximize the temperature difference hence the difference in resistance between two filaments (thin tungsten wires). The large surface-to-mass ratio

permits a fast equilibration to a steady state. The temperature difference between the reference and the sample cell filaments is monitored by a Wheatstone bridge circuit.

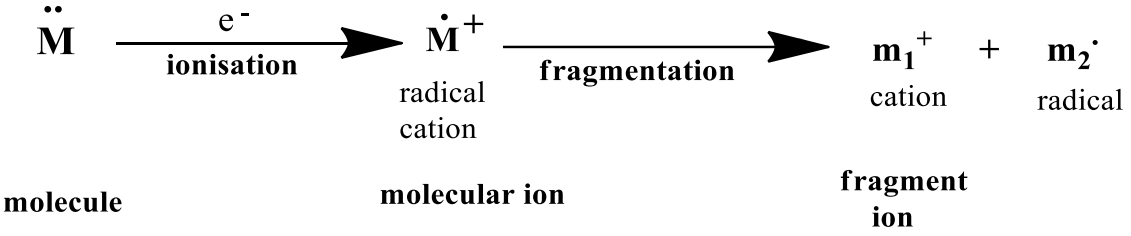
2.8.3.5.3 Electron Capture Detector (ECD)

This detector consists of a cavity that contains two electrodes and a radiation source that emits radiation (^{63}Ni, ^{3}H). The collision between electrons and the carrier gas (for example methane plus an inert gas) produces a plasma-containing electrons and positive ions. If a compound is present that contains electronegative atoms, those electrons will be "captured" to form negative ions and the rate of electron collection will decrease. The detector is extremely selective for compounds with atoms of high electron affinity (10^{-14} g/s), but has a relatively small linear range. This detector is frequently used in the analysis of chlorinated compounds which include pesticides (herbicides, insecticides) and polychlorinated biphenyls for which it exhibits a very high sensitivity.

2.8.3.5.4 Mass Spectrometer

The mass spectrometer (MS) helps to identify compounds based on their fragmentation patterns after being separated from each other at the GC.

Mass spectroscopy deals with the study of the charged molecules and fragment ions produced from a sample exposed to ionizing conditions. The relative intensity spectrum results from the correlation of the ions with their mass to charge ratio.



Scheme 1: Ionisation and fragmentation of a molecule in a mass spectrometer

Samples are vaporized and bombarded by a beam of electrons and unstable radical cations are formed which decompose to smaller fragments as illustrated in Scheme 1. The positively charged ions are accelerated towards a negatively charged plate, passed through analyzer in a magnetic field where they are deflected at different rates depending on their m/z ratios. The schematic diagram of a simple mass spectrometer is shown in Figure 26.

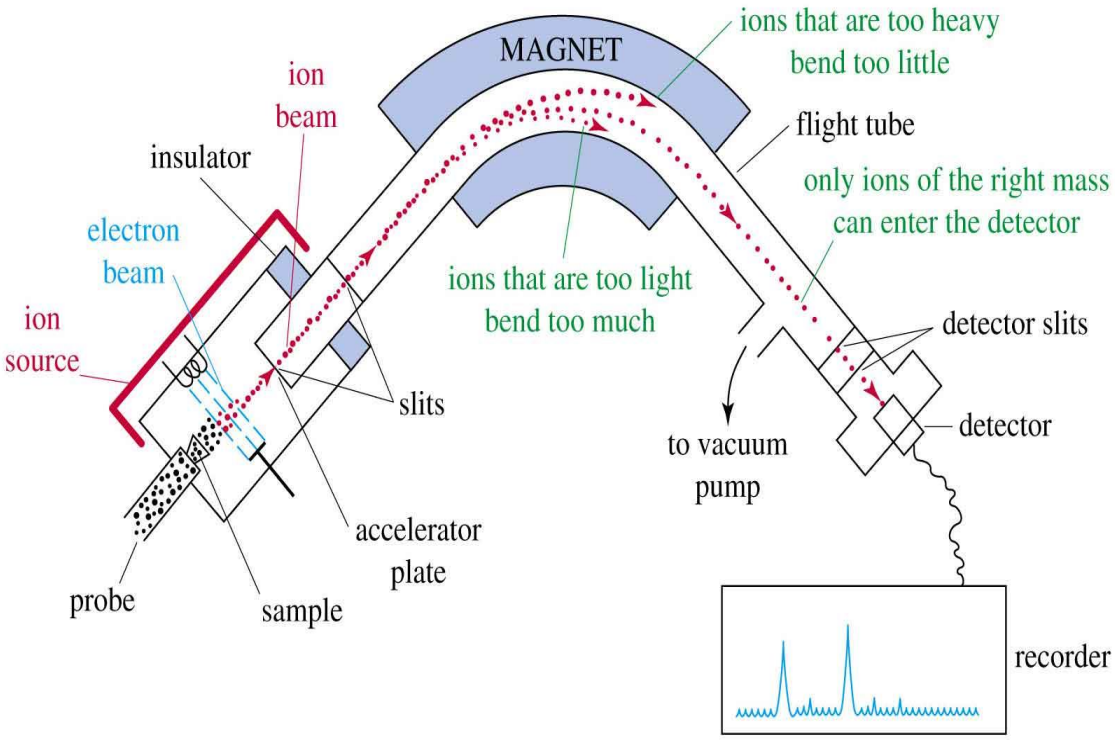


Figure 26: A schematic diagram of a mass spectrometer

2.8.4 Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas Chromatography-Mass Spectroscopy is an integrated composite analysis combining gas chromatograph (GC) which is excellent in its ability for separation with mass spectrometer (MS) which is ideal for the identification and elucidation of structures of separated components. GC-MS is a hyphenated analytical technique which refers to an on-line combination of a chromatographic separation technique with a sensitive and element-specific spectroscopic detector. GC can separate volatile and semi-volatile compounds with great resolution, but cannot identify them. MS provides detailed structural information on most compounds such that they can be exactly identified and with that, both qualitative and quantitative information about the samples can be obtained using GC-MS (Figure 27).

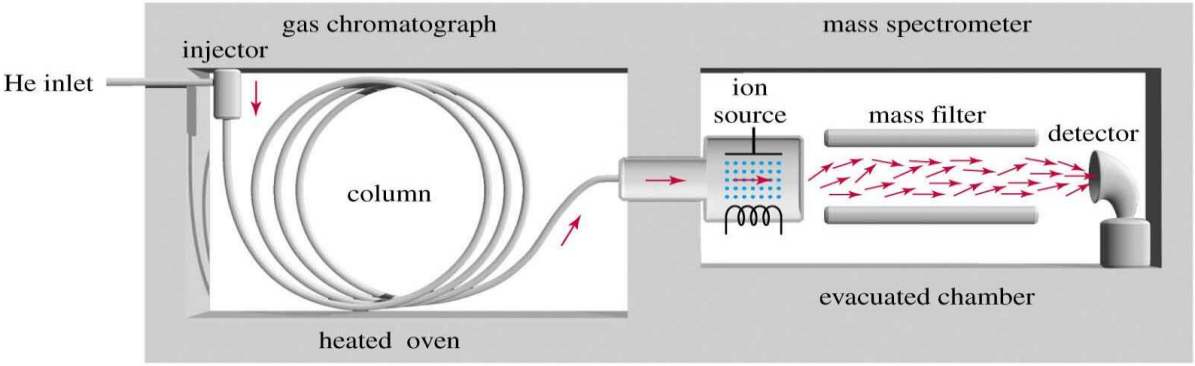


Figure 27: Schematic diagram of GC-MS

For an analysis, the sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas, usually helium. The sample flows through the column and the mixture comprising of the compounds of interest are separated by virtue of their relative interaction with the coating of the stationary phase and the mobile phase. The column terminates at an interface where the carrier gas molecules are removed. The components now move into the ion chamber of the MS where they are converted to ions and detected according to their mass to charge (m/z) ratio. The components can be identified by the interpretation of the recorded mass spectra and the identifications confirmed by using

the library search facilities of the data system. A list of possible substances is produced with a percentage that indicates how well the spectra are matched against the unknown.

A mass spectrum is a plot of an intensity agaist m/z (mass-to-charge ratio) representing a chemical analysis. Hence, the mass spectrum of a sample is a pattern representing the distribution of ions by mass in the sample. A typical mass spectrum of methyl 6, 9, 12-octadecatrienoate is shown on Figure 28.

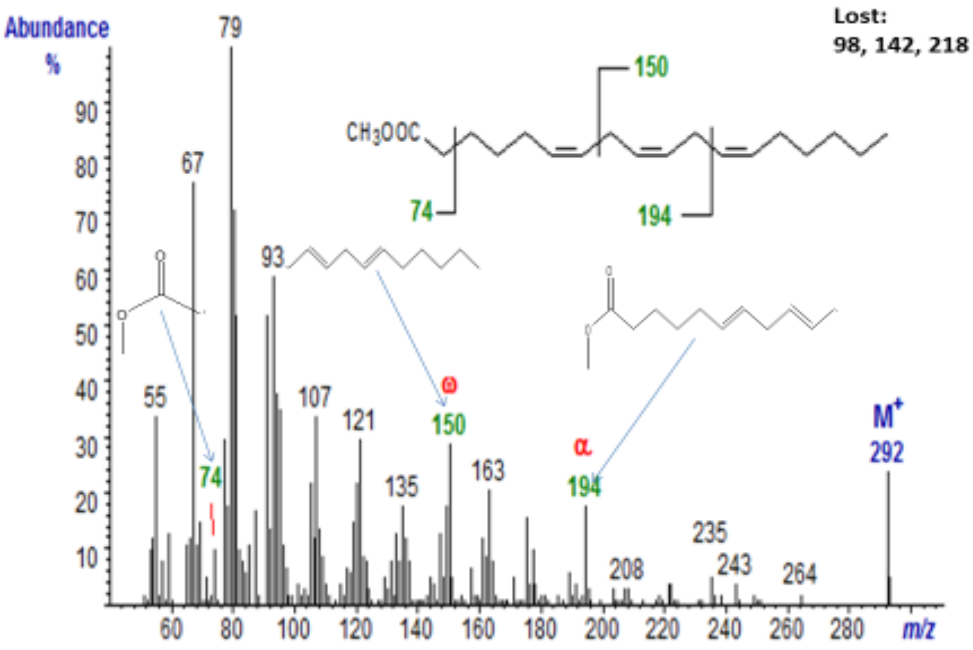


Figure 28: Mass specrum of methyl 6, 9, 12-octadecatrienoate (γ-linolenate); a polyunsaturated fatty acid methyl ester (Holman and Rahm, 1971)

2.8.5 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is characterized by the use of high pressure to push a mobile phase solution through a column of stationary phase allowing separation of complex mixtures with high resolution. It is primarily a quantitative method of analysis for non-volatile organic compounds. HPLC instruments consist basically of a reservoir of mobile phase, a pump, an injector, a separation column and a detector.

The end requirement for the pure compounds is what determines the technique used which is based on the size and scale of the chromatographic column. If microgram quantities of compounds are needed for initial bioassay screening, then purification can be carried out using analytical-scale HPLC systems, where the column internal diameter (i.d.) is usually around 4.6 mm. Greater quantities are usually needed for structure elucidation purposes in which case, a laboratory-scale prep HPLC system is required to isolate the milligram quantities needed and the column internal diameters usually range from 10 to 100 mm. If gram quantities are called for, then typically pilot plant-scale prep HPLC systems are needed (column i.d.>100 mm). The theory behind the isolation process is essentially the same in each case (Latif and Sarker, 2012).

Prep HPLC has high consistency and smaller particle size of the stationary phase (3 to 10 mm) hence better separating power (or resolution) when compared to other ‘‘lower pressure’’ column chromatographic systems. The small particle size results in having to use high pressures (up to 3–4000 psi) to push the mobile phase through the system. However, the high surface area available for the solutes to interact with the stationary phase results in a chromatography with high powers of resolution that are necessary for purifying complex natural product mixtures. The materials and methods used for isolating natural products by prep HPLC also depend upon the type of compound that is encountered in the extract, which

in turn is dependent upon the extraction procedure. A polar extract of a plant obtained using aqueous ethanol will differ substantially in compounds encountered than if the same plant was extracted with n-hexane. Therefore, polarity of the compound mixture is a major deciding factor as to which mode of prep HPLC method is to be used.

Prep HPLC isolation or purification of natural products typically uses one of the following four chromatographic modes: normal-phase, reversed-phase, gel permeation chromatography, and ion exchange chromatography. The modes are determined by the stationary phase, column used, and the solvents utilized for elution. For each separation mode, compatibility must exist between the sample mixture (with the mobile phase) and the stationary phase.

Normal-phase chromatography uses a polar stationary phase (usually silica) and less polar (non aqueous) eluting solvents. Compounds are separated by adsorption onto the surface of the polar stationary phase as they elute down the column and the affinity they have to the eluting nonpolar solvent. In general, the more polar the compound, the more likely it is to be adsorbed onto the stationary phase, and less polar compounds will be eluted first from the column. Increasing the polarity of the eluting solvent reduces elution time. Normal-phase HPLC is best suited to lipophilic compounds, long chain alkane derivatives, or where the mixture of interest is sparingly soluble in aqueous conditions. The eluants used in normal phase HPLC are usually mixtures of aliphatic hydrocarbons (n-hexane, n-heptane), halogenated hydrocarbons (chloroform, dichloromethane), more polar oxygenated hydrocarbons (diethyl ether, ethyl acetate, acetone), or hydroxylated solvents such as isopropanol. Care must be taken to control the aqueous content of the solvents as water deactivates silica causing a breakdown in the separation (Latif and Sarker, 2012).

Reversed-Phase HPLC is the reverse of normal-phase HPLC, whereby the stationary phase is more nonpolar than the eluting solvent. Examples of reversed stationary phases are C_{18}, C_8

and polymeric polystyrene divinyl benzene. Silica-based reversed-phase sorbents are also called ‘‘bonded-phase’’ materials, whereby the silica particles are derivatized with alkylsilyl reagents. The degree of silanization (or carbon loading) can result in columns having substantially varying chromatographic characteristics and in some cases several columns may be used for separating different mixtures (Eurby and Petersson, 2003). The eluant used in reversed-phase HPLC commonly comprises a mixture of water and miscible organic solvents, usually acetonitrile (MeCN), methanol (MeOH), or tetrahydrofuran (THF). In addition, buffers, acids, or bases may be added to suppress compound ionization or to control the degree of ionization of free unreacted silanol groups to reduce peak tailing and improve chromatography. Reversed-phase HPLC is used for the purification and isolation of most classes of natural products. Because of this, it is usually the first technique used when analysing and attempting to purify compounds from a complex mixture especially when the identity of the compounds of interest is unknown. The instrumentation processes for both normal and reversed-phase HPLC are the same (Latif Z. 2005).

2.8.5.1 HPLC Instument Overview

The instrumentation process of HPLC system is represented with a schematic diagram in Figure 25. A reservoir holds the solvent which is the mobile phase. A high-pressure is used to generate and deliver a specified flow rate of the mobile phase, typically milliliters per minute. An injector which is an autosampler is able to introduce the sample into the continuously flowing mobile phase stream and the sample is carried along into the HPLC column. The column contains the stationary phase which is the chromatographic packing material needed to effect the separation. The separated compounds are recognised by the detector like bands as they elute from the HPLC column. The detector is connected to the computer data station which is the system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantify the concentration of

the sample constituents. The mobile phase exits the detector and can be sent to waste, or collected as desired. The separated compound band enables one to collect the fraction of the eluate containing the purified compound for further study (Vunguturi and Ali, 2015).

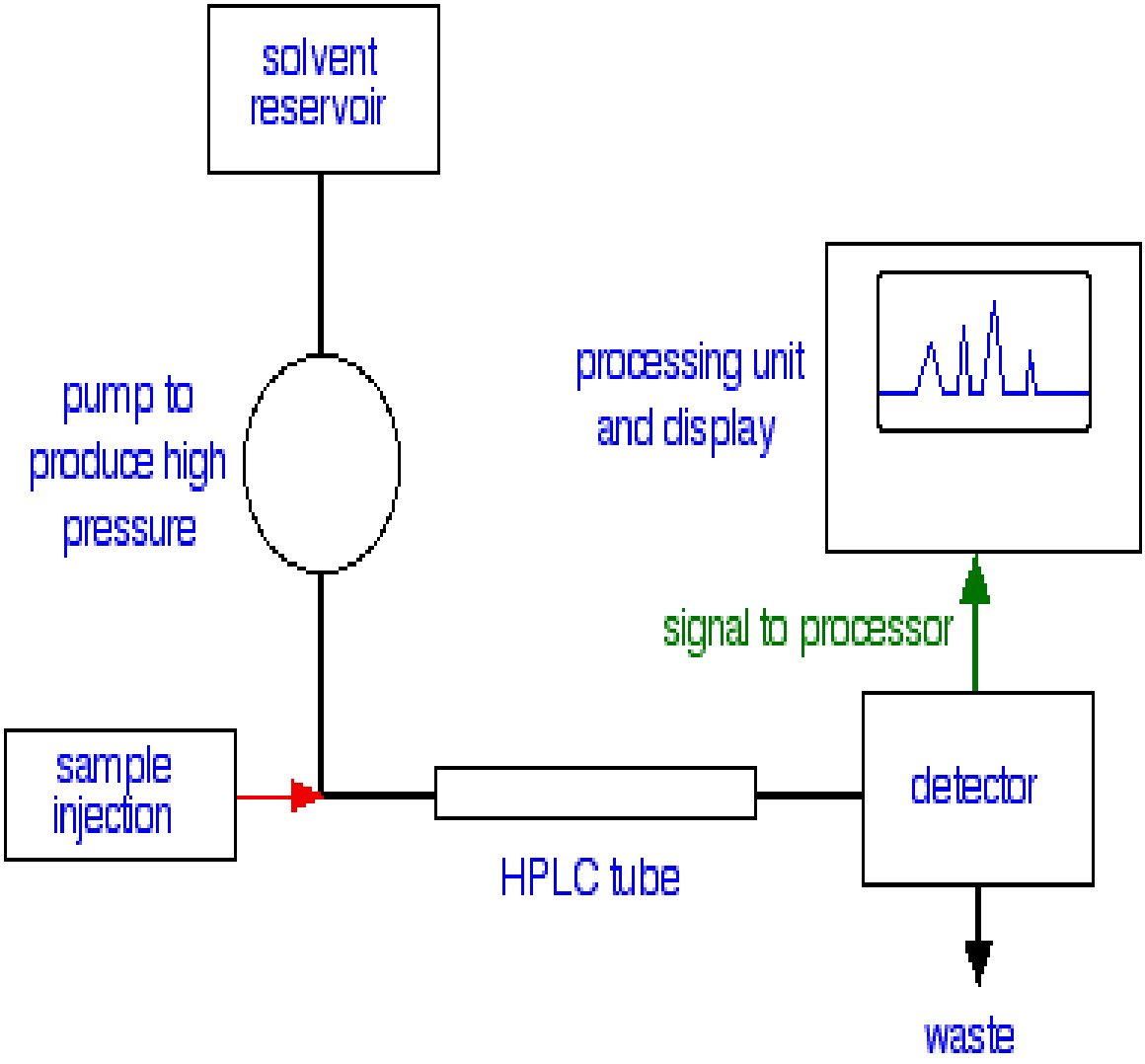


Figure 29: Schematic diagram of HPLC system

2.8.5.2 HPLC Detectors

Since the characteristics of compounds in a sample can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector (ELSD). The most powerful approach is the use of multiple detectors in series. For example, a UV and ELSD detector may be used in combination with a mass spectrometer (MS) to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. Some detectors used in HPLC, their attributes and sensitivities are shown in Table 6.

Table 6: Common HPLC detectors, their attributes and sensitivities (Satinder and Michael, 2005)

Detector	Analyte/attributes	Sensitivity
UV/Vis absorbance	Specific: compounds with UV chromophores	ng
Diode array	Specific: same as UV/Vis detectors also provides UV spectra	ng
Fluorescence	Specific: compounds with native fluorescence or with fluorescent tag	fg-pg
Refractive index	Universal: polymers, sugars, triglycerides, organic acids, excipients	0.1-10~tg
Evaporative light-scattering	Universal: non-volatile or semi-volatile compounds, compatible to gradients	Low ng
Electrochemical	Specific: electro-active compounds	pg
Conductivity	Specific: anions and cations, organic acids, surfactants	ng ppm – ppb
Radioactivity	Specific, radioactive-labelled compounds	Low levels
Mass spectrometry	Both universal and specific, definitive identification	fg-pg-ng

CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials and Methods

The solvents which include hexane, ethyl acetate, butanol, methanol and dichloromethane (DCM) were obtained from Sigma-Aldrich chemicals as both analytical and HPLC grade.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Fluka, UK. Quercetin was obtained from Avocado Research Chemicals Ltd. Lancashire. Others include: Dimethyl sulfoxide (DMSO) which was obtained from Sigma-Aldrich, Iso-Sensitest broth with agar by Oxoid and 1/4 ringers tablet from Lab M Ltd. Lancashire, UK. Saline tablets were from Scientific Laboratory Supplies, Nottingham, UK. Resazurin tablets, multichannel pipettes and sterile tips of various sizes were from Fischer Scientific. Ciprofloxacin was obtained from Sigma-Aldrich. Millipore filters and microtiter plates were from Sarstedt while Vortex mixer was from Stuart Scientific. Soxhlet extraction was carried out using soxhlet apparatus from Barnstead Electrothermal, UK. Sample clean-up was with carbon eighteen (C_{18}) solid phase extraction (SPE) cartridge Strata X from Phenomenex. GC-MS analyses of the essential oil samples from all the plants with the exception of *Pterocarpus osun* were carried out on GC Model HP 7890A and MS model 5975C VLMSD by Agilent Technologies Ltd. The GC-MS analysis of *P. osun* essential oil was on GC-MS model QP-2010 ultra Shimadzu Spectrometer. The absorbances of the extracts and fractions were measured using UV-VIS Spectrophotometer by Perkin Elmer (Lambda XLS). All liquid chromatographic separations were performed on Dionex Ultimate 3000 series HPLC and the eluting peaks were detected using Chromeleon software. A C_{18} Semiprep Phenomenex Column (5 micron particle size, 150 mm x 10 mm I.D.) monitored by photodiode array detector was used. MS analysis of the isolated quercetin was performed on a Quattro II triple quadrupole mass spectrometer by

Waters, UK. The antibacterial assay was carried out at the Biomedical and Microbiology Department, University of Wolverhampton, UK.

3.2 Flowcharts showing the methodology

The flowcharts showing the methodologies for the extractions and analyses of essential oils and methanolic extracts are shown in Figures 30 and 31 respectively.

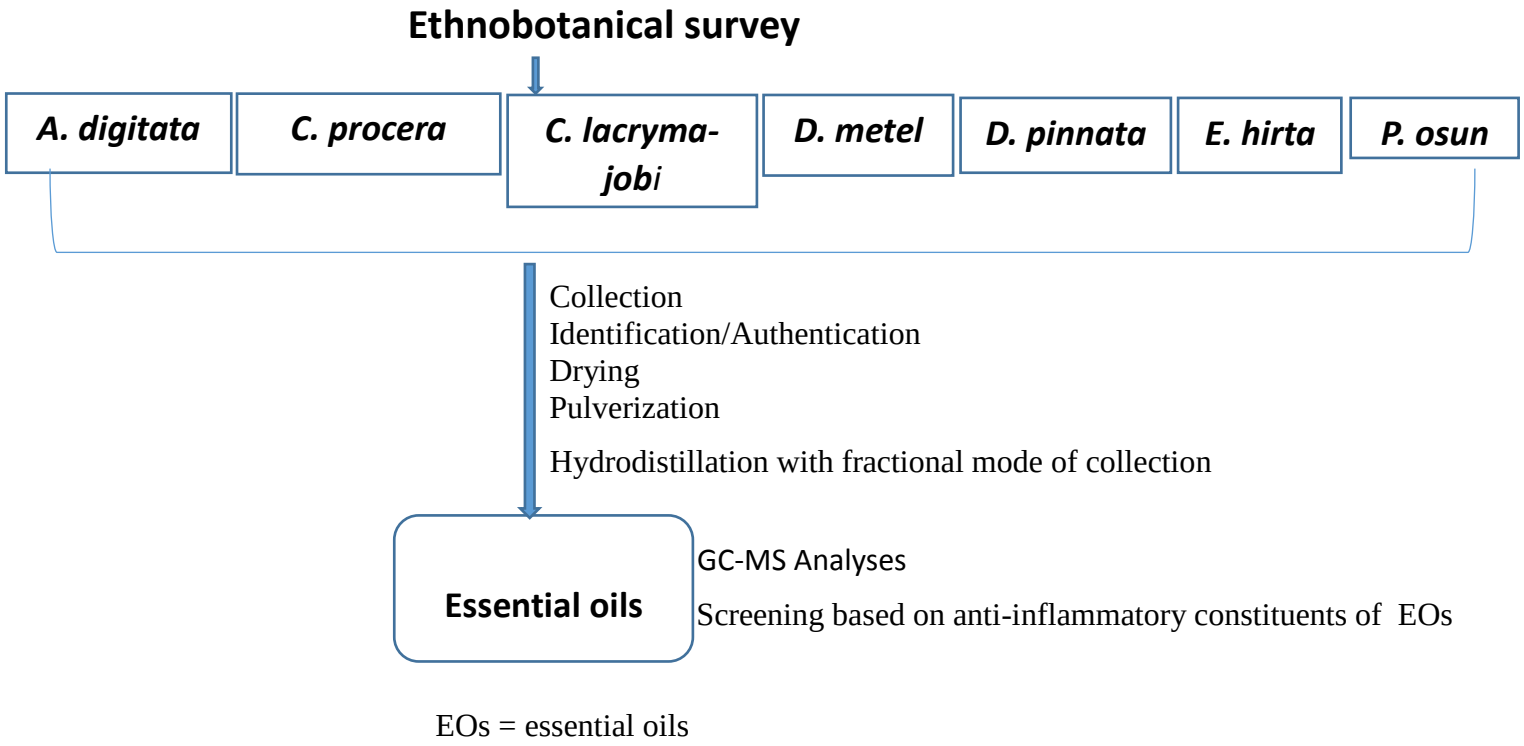
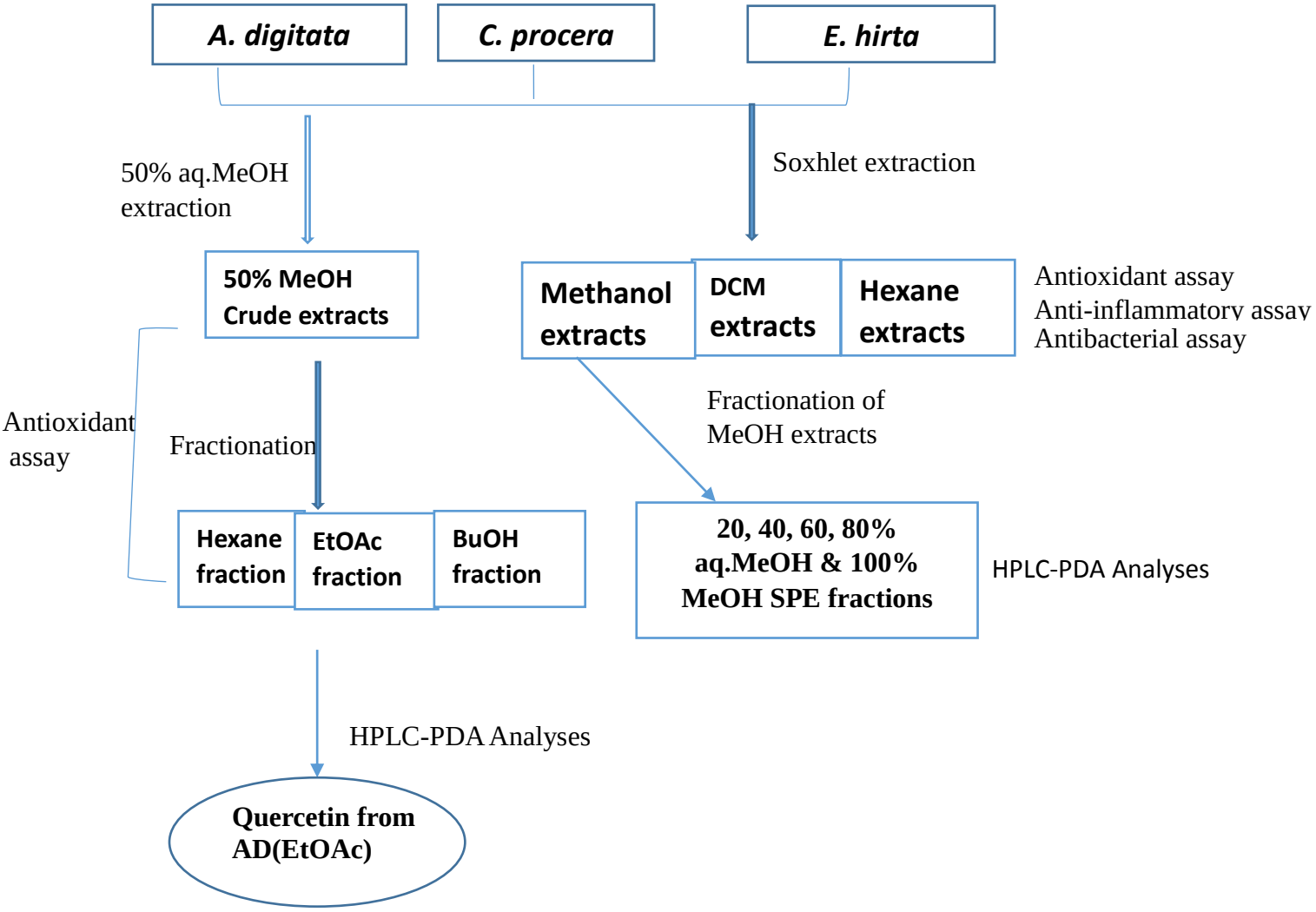


Figure 30: Flowchart showing the methodology for the extractions and analyses of essential oils



MeOH = methanol; BuOH = butanol; EtOAc = ethylacetate; SPE = solid phase extraction
AD(EtOAc) = ethylacetate fraction of *Adansonia digitata*

Figure 31: Flowchart showing the methodology for the extractions and analyses of the methanolic plant extracts

3.3 Plant Collection and Processing

A list of medicinal plants used for the management of asthma was prepared based on: review of books on medicinal plants and applications, journal publications and information from Taxonomists and Practitioners of Alternative medicine. The plant materials were collected, identified and authenticated by a Botanist, Mr. Oyetola Oyebanji of Botany Department, University of Lagos. *Adansonia digitata* (leaves), *Calotropis procera* (leaves), *Datura metel* (leaves) and *Euphorbia hirta* (aerial parts) were registered and sample specimens deposited in the herbarium at Forestry Research Institute of Nigeria (FRIN), Ibadan while those for *Coix lacryma-jobi* (leaves), *Deinbollia pinnata* (leaves) and *Pterocarpus osun (*roots) were deposited at University of Lagos Herbarium. All the plants were assigned with voucher numbers as shown in Table 7. All the plant samples were air-dried in a dust–free environment for an average of 2 weeks except for the roots of *Pterocarpus osun* which were dried for 4 weeks.

Table 7: Authentication details of the medicinal plants under study

S/N	Plants	Date and season of collection	Place of collection	Voucher no.
1.	*Euphorbia hirta* L.	July 2007 (rainy season)	Mushin market Lagos	FHI 107760
2.	*Calotropis procera* (Aiton) W.T. Aiton	August 2007 (rainy season)	Mushin market Lagos	FHI 107882
3.	*Adansonia digitata* L.	April 2009 (rainy season)	Water front close to Botanical garden, University of Lagos	FHI 108213
4.	*Datura metel* L.	August 2009 (rainy season)	Ikire, Osun State	FHI 103360
5.	*Coix lacryma-jobi* L.	July 2011 (rainy season)	Ikire, Osun State	LUH 3390
6.	*Deinbollia pinnata* (Poir.) Schumach. & Thonn.	July 2011 (rainy season)	Ikire, Osun State	LUH 3391
7.	*Pterocarpus osun* Craib	July 2011 (rainy season)	Ikire, Osun State	LUH 3158

3.4. Hydrodistillation of Essential Oils

The essential oil (E.O.) samples from each of the plant parts were obtained by hydrodistillation using Clavenger apparatus. Batches of 100 g of pulverized plant materials were mixed with 3 L of distilled water in a 5 L round-bottomed flask and heated using a heating mantle. The vapours of the E.O. were condensed and collected into hexane. In one mode, fractions of E.O. were collected hourly over a 4 hour period whereas in another mode, a single collection was made over a 4 hour period. The hexane in the extracts was evaporated at room temperature to concentrate the samples which were then kept in the refrigerator till the time for analyses. The essential oil samples were diluted with hexane and dried over anhydrous sodium sulphate to remove any trace of water prior to analyses on Gas Chromatograph–Mass Spectrometer (GC-MS).

3.5 Gas Chromatography – Mass Spectroscopy (GC-MS) analyses of the essential oil samples from the plants under study

About 1µL of each of the essential oil samples was injected automatically into the gas chromatograph which was fitted with HP-5MS capillary column 30 m x 320 µm x 0.25 µm having 5% phenylmethyl siloxane as the stationary phase. Mass spectra were recorded using ionisation energy of 70 eV. Identification was made possible by National Institute of Standards and Technology (NIST) with the Chem-Office software and library of the MS. The temperature programs for the GC-MS analyses of the essential oils from the medicinal plants under study are as shown in Table 8.

Table 8: Medicinal Plants with their temperature programs for GC-MS Analyses

S/N	Plants	Temperature Programs
1.	*Adansonia digitata* L.	50 °C (1 min), increased at 2.5 °C /min to 230 °C.
2.	*Calotropis procera* (Aiton) W.T. Aiton	120 °C (6 min), increased at 5 °C /min to 320 °C (6 min).
3.	*Coix lacryma-jobi* L.	100 °C (2 min), increased at 5 °C /min to 140 °C (2 min), further increased at 2.5 °C /min to 240 °C (8 min).
4.	*Datura metel* L.	50 °C (1 min), increased at 2.5 °C /min to 230°C.
5.	*Deinbollia pinnata* (Poir.) Schumach. & Thonn	100 °C (2 min), increased at 3.5°C / min to 150 °C (2 min), further increased at the same rate to 180 °C (1 min) and thereafter increased at 4 °C / min to 250 °C (1 min).
6.	*Euphorbia hirta* L.	120 °C (5 min), increased at 5 °C /min to 230 °C (5 min).
7.	*Pterocarpus osun* Craib	60 °C (2 min), increased at 9 °C /min to 290 °C

Based on the results of the analyses of the essential oil samples, three plants - *Adansonia digitata* (leaves) [AD], *Euphorbia hirta (*aerial parts) [EH] and *Calotropis procera (*leaves) [CP] were chosen for further investigations.

3.6 The 50% Aqueous Methanol (aq.MeOH) Extraction and Fractionation

This procedure was carried out on the selected plant materials: *Adansonia digitata* (leaves), *Euphorbia hirta* (aerial parts) and *Calotropis procera (*leaves).

The 50% aq.MeOH extraction was according to a protocol by Okiei *et al.*, 2011. A batch of 200 g of pulverised sample was added to 3 L MeOH/H_2O (1:1) at room temperature with intermittent and gentle stirring over 72 hours. The mixture was filtered and the residue extracted with 500 mL of the solvent over 24 hours. The combined filtrate was concentrated in a rotary evaporator and the extract obtained dried under a jet of air at room temperature.

The dried 50% aq.MeOH extract was then dissolved in distilled water and fractionation was performed sequentially using non-polar solvent (hexane), medium polar solvent (ethyl acetate) and polar solvent (butanol). The solvents were evaporated to obtain the fractions.

3.7. Soxhlet Extraction

About 100 g each of dried and pulverised plant parts of *A. digitata* (leaves), *E. hirta (*aerial parts) and *C. procera* (leaves) were extracted sequentially for 10 cycles with n-hexane, dichloromethane (DCM) and methanol (MeOH) using soxhlet extractor. The extracts obtained were filtered using Whatman filter paper (15 cm) after which the filtrates were concentrated on a rotary evaporator and three different extracts hexane, DCM and MeOH extracts obtained. This procedure was carried out in triplicate.

3.8 Solid Phase Extraction (SPE)

SPE was used as a clean-up process for the soxhlet methanol extracts before liquid chromatographic analysis. The concentrated methanol extracts were cleaned up using a C_{18} solid phase extraction cartridge previously conditioned with 50 mL of methanol followed by equilibration with 100 mL of distilled water. About 2 g of the MeOH extract of each of the plant materials was mixed with 2 g of silica (70 mesh) and loaded into the cartridge. The samples were eluted each with 200 mL stepwise gradient solvent system of 20, 40, 60, 80% aq. MeOH and 100% MeOH. The resulting fractions were then concentrated, dried and stored in a refrigerator.

3.9 High Performance–Liquid Chromatographic (HPLC) Analyses, Bioassay – guided isolation of active compounds and Mass spectroscopy

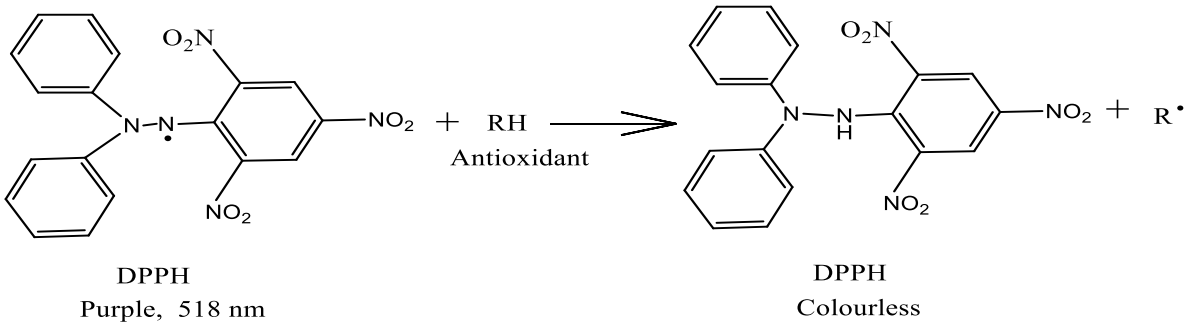
High Performance–Liquid Chromatographic separation was performed on all the fractions of *Adansonia digitata* (leaves), *Calotropis procera (*leaves) and *Euphorbia hirta (*aerial parts). Chromatographic separations were performed on Dionex Ultimate 3000 series HPLC systems equipped with a pump, autosampler, photodiode - array detector (220 – 331nm) and a degasser. The column compartment temperature was 25 ^{0}C and the eluting peaks were detected using Chromeleon software. The injection volume was maintained at 10 µL for all the investigations and the mobile phase consisted of water A: MeOH / B (70:30) with a flow rate of 2.0 mL/min. A C_{18} column was used and the samples were eluted with a linear gradient 0 min, 70% A/30% B; 30 min, 0% A -100% B and 43 min, 70% A/30% B monitored by the Photo–diode array detector with which UV-Vis spectra of the extracts/fractions were obtained. The isolation of an antioxidant was done using the same conditions and was bioactivity-guided. The antioxidant activity of the extracts and fractions of *A. digitata, C. procera* and *E. hirta* were first determined and ethyl acetate fraction of *A. digitata* exhibited the highest antioxidant activity among the extracts obtained using different solvents hence was used for the isolation. Specifically, the analyses for the isolation process were performed on crude ethyl acetate fraction of *A. digitata*, the isolated compound and quercetin standard. The compound was further confirmed using the mass spectral data and those of the standard compound under the same conditions.

3.10 *In vitro* Bioassays of the plants

3.10.1 Antioxidant Assay

The antioxidant activities of the extracts/fractions from Soxhlet, SPE and 50% aq.MeOH extractions of *Adansonia digitata* (leaves), *Euphorbia hirta (*aerial parts) and *Calotropis*

*procera (*leaves) based on their scavenging activities on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, were determined. DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up as illustrated in Scheme 2.



DPPH
Purple, 518 nm

DPPH
Colourless

Scheme 2: Reaction of DPPH free radical with an antioxidant

The antioxidant activities of the extracts and fractions on DPPH radicals were assessed. About 1mL of the extracts (10 mg/mL) which was serially diluted one fold (0.0001 – 1 mg/mL) in MeOH was mixed with 1 mL of DPPH (0.08 mg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. A control was prepared with 1 mL of the DPPH and 1 mL of MeOH. Quercetin was used as positive control. The absorbance of the mixture was measured at 518 nm against the blank (1 mL MeOH) with UV-VIS Spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The test was done in duplicates and the average absorbance obtained.

The percentage inhibitions of the free radicals due to the antioxidant activities of the extracts/fractions were calculated using the formula,

$$\% \text{ Inhibition } = \frac{\text{ABS}_{\text{ blank}} - \text{ABS}_{\text{ sample}}}{\text{ABS}_{\text{ blank}}} \times 100$$

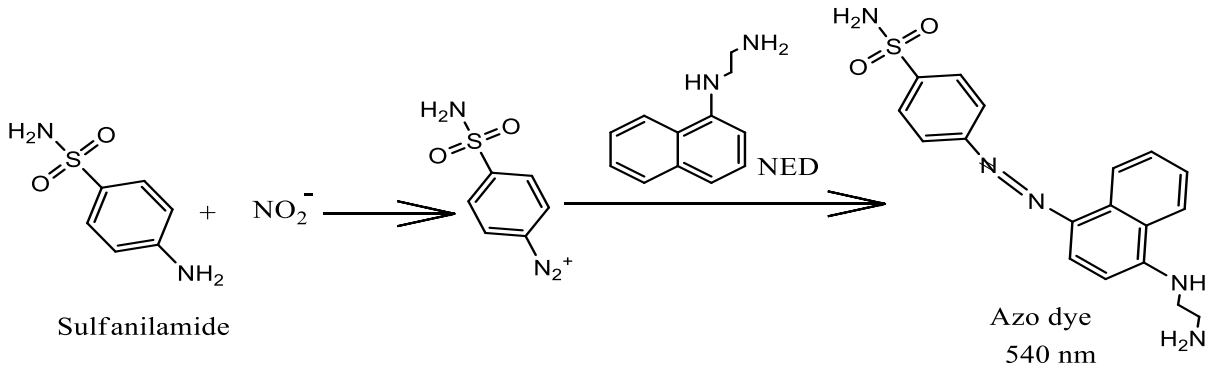
ABS = Absorbance

Equation 2: Calculation of Percentage Inhibition

The linear regression plots for those samples with up to 50% Inhibition Concentration ($1C_{50}$) were obtained using further one-fold dilution and from the regression equations derived, the actual $1C_{50}$ values were calculated.

3.10.2 Anti-inflammatory Assay

The anti-inflammatory activities of the methanol extracts of *A. digitata* (leaves), *E.hirta* (aerial parts) and *C. procera* (leaves) were assessed. The test was carried out using the method reported by Olajide *et al.,* 2007 which was based on the quantification of nitrite accumulation in RAW 264.7 murine macrophages. Cells were seeded in 96-well plates (200 μL/well), cultured for 2 days and incubated with lipopolysaccharides (LPS) (100 ng/mL) in the presence of the extracts (25–100 μg/mL) for 24 h. As a parameter of NO production, nitrite concentration was assessed in cell supernatants by the Griess reaction whereby 50 μL of cell culture supernatant was added to 50 μL of 1% sulfanilamide in 5% H_3PO_4. Then, 50 μL of 0.1% N-(1-naphthyl) ethylenediamine (NED) dihydrochloride in H_2O was added to the mixture in a 96-well plate. The experiments were performed in triplicate and spectrophotometric measurements were carried out at 540 nm. Nitrite concentrations in the supernatants were determined by comparison with a sodium nitrite standard curve. Scheme 3 is an illustration of the Greiss reaction.



NED = N-(1-naphthyl) ethylenediamine

Scheme 3: The Griess reaction

3.10.3 Antibacterial Assay

The assay was carried out on soxhlet extracts of *Adansonia digitata, Calotropis procera and Euphorbia hirta* using the modified colorimetric Resazurin-based Microtiter Dilution Assay (RMDA) adopted by Sarker *et al*., 2007. Antibacterial activities of extracts were tested against two gram positive bacteria (*Staphylococcus aureus* NCTC 6571 and *Bacillus cereus* NCIMB 3329) and three gram negative bacteria (*Escherichia coli* NCTC 10418, *Acinetobacter baumannii* NCIMB 12457 and *Pseudomonas aeruginosa* NCTC 10662).

The resazurin solution was prepared by dissolving one tablet of resazurin in 10 mL of sterile distilled water. A sterile 96-well plate was prepared under aseptic conditions. A volume of 100µL of test extracts (10 mg/mL) in 10% (v/v) DMSO was pipetted into the first row of the plate. To all other wells 50 µL of normal saline was added. Serial dilutions were performed such that each well had 50µL of the test material in serially descending concentrations. To each well 10µL of resazurin indicator solution, 30 µL of isosensitest broth and finally 10µL of bacterial suspension was added. Each plate was wrapped loosely with cling film to ensure that the bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad spectrum antibiotic as positive control (Ciprofloxacin in serial dilutions), a column with all solutions with the exception of the test compound and a column with all solutions with the exception of the bacterial suspension adding 10µL of normal saline instead.

The plates were prepared in triplicate and incubated at 37˚C for 24 hours. The colour change was then assessed visually. The colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the minimum inhibitory concentration (MIC) value. The average of three values was calculated and that was the MIC for the test material and bacterial strain.

3.11 Statistical Analyses

Data were calculated as means ± standard deviation. Pearson's corelation analysis and T – test were carried out using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corporation.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant Collection, Processing and Extraction of Essential Oils

Some of the physical properties (% moisture, % yield and colour) of the essential oil samples obtained from the hydrodistillation of the leaves of *Adansonia digitata, Calotropis procera, Coix lacryma-jobi, Datura metel, Deinbollia pinnata,* aerial parts of *Euphorbia hirta* and the roots of *Pterocarpus osun* are presented on Table 9.

The percentage moisture in the fresh plants was in the range of 54.86 – 74.57%. The yield of the essential oil was in the range of 0.05 – 0.15%. These values and the colour of each essential oil sample are presented in Table 9.

Table 9: Moisture contents of plant parts, yield and colour of essential oils obtained from the medicinal plants investigated

S/N	Medicinal Plants	% Moisture in Fresh Plants	%Yield of Essential oils	Colour
1.	*Adansonia digitata*	62.07	0.15	Yellow
2.	*Calotropis procera*	70.05	0.05	Colourless
3.	*Coix lacryma-jobi*	57.37	0.10	Colourless
4.	*Datura metel*	73.54	0.08	Yellow
5.	*Deinbollia pinnata*	65.27	0.05	Light yellow
6.	*Euphorbia hirta*	74. 57	0.10	Light brown
7.	*Pterocarpus osun*	54.86	0.10	Colourless

4.2 GC-MS analyses of the essential oil samples from the plants under study

The results of the GC-MS analyses of different essential oil samples from the leaves of *A. digitata, C. procera, C. lacryma-jobi, D. metel, D. pinnata,* aerial parts of *E. hirta* and roots of *P.osun* are presented in Figures 32 – 54 and Tables 10 – 16.

4.2.1 GC-MS analyses of the essential oil samples from the leaves of *Adansonia digitata*

The chromatograms obtained from the GC-MS analyses of the essential oil samples obtained from the 1st, 3rd, 4th hourly fractions and 4 hrs single collection of *A. digitata* are shown in Figures 32-35 and the retention times, names, formulae, classes and percentage abundances of the various constituent compounds are presented in Table 10.

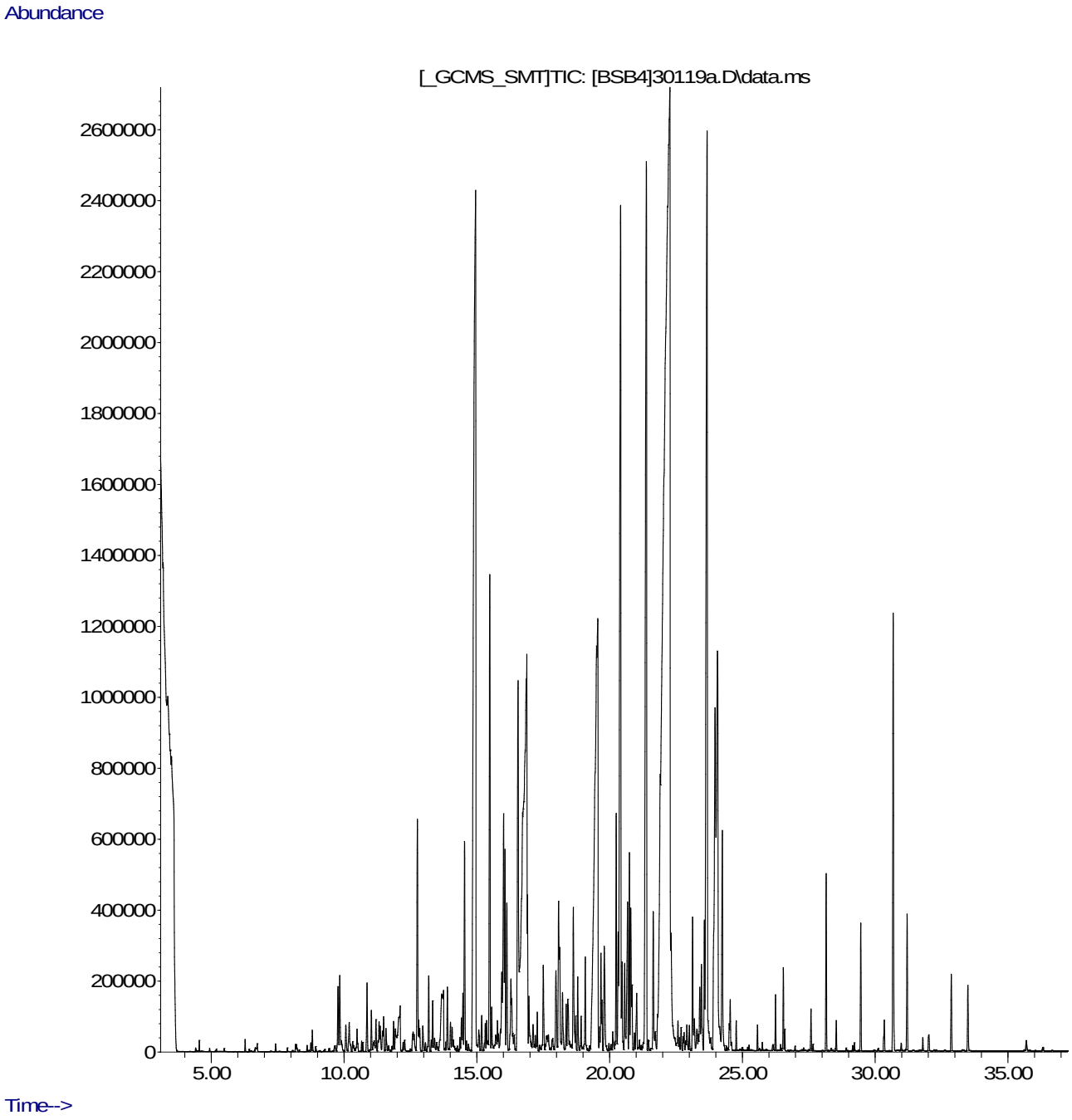


Figure 32: Total Ion Chromatogram (TIC) of *Adansonia digitata* leaves essential oil collected after the 1st hr

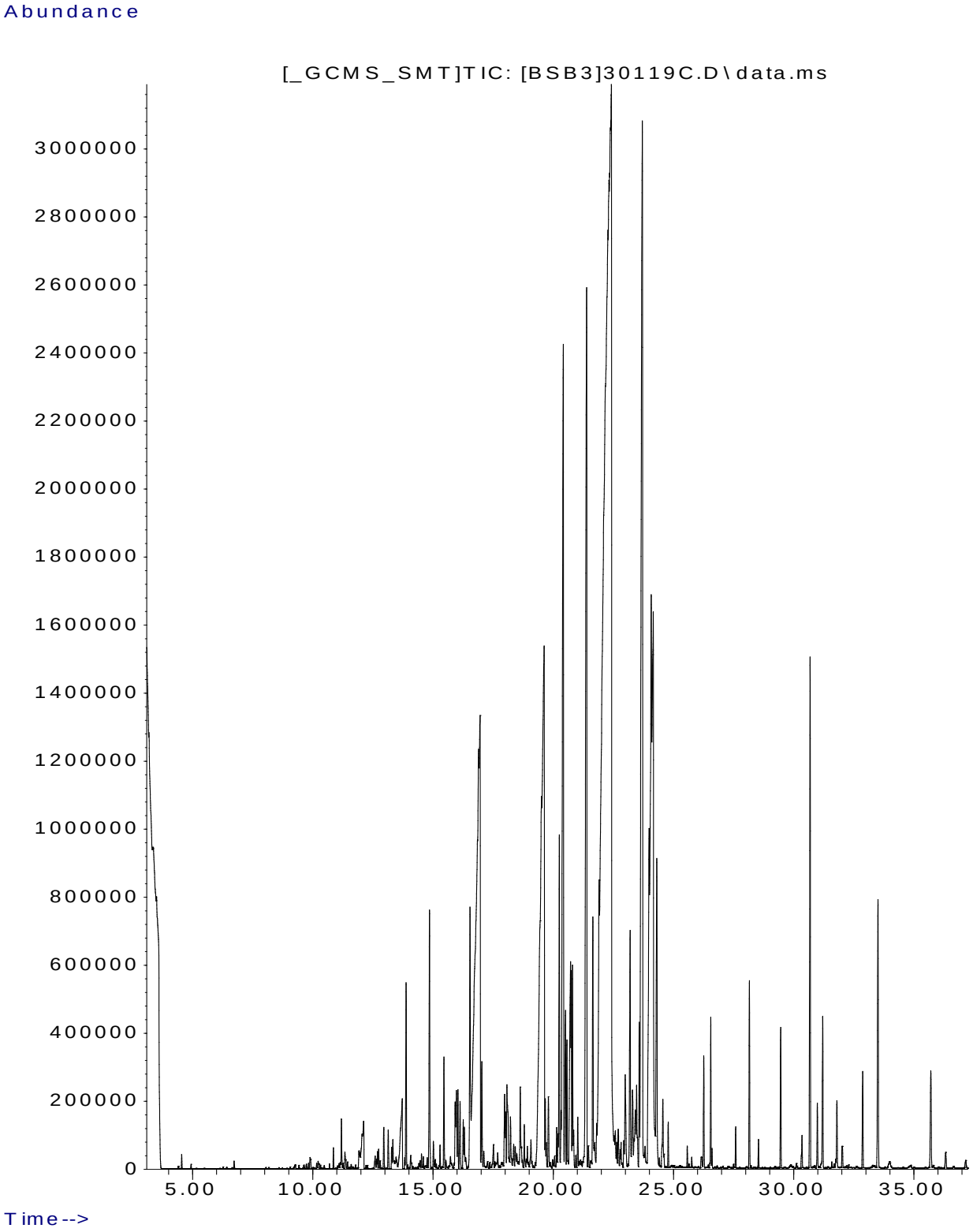


Figure 33: TIC of *Adansonia digitata* leaves essential oil collected after the 3rd hr

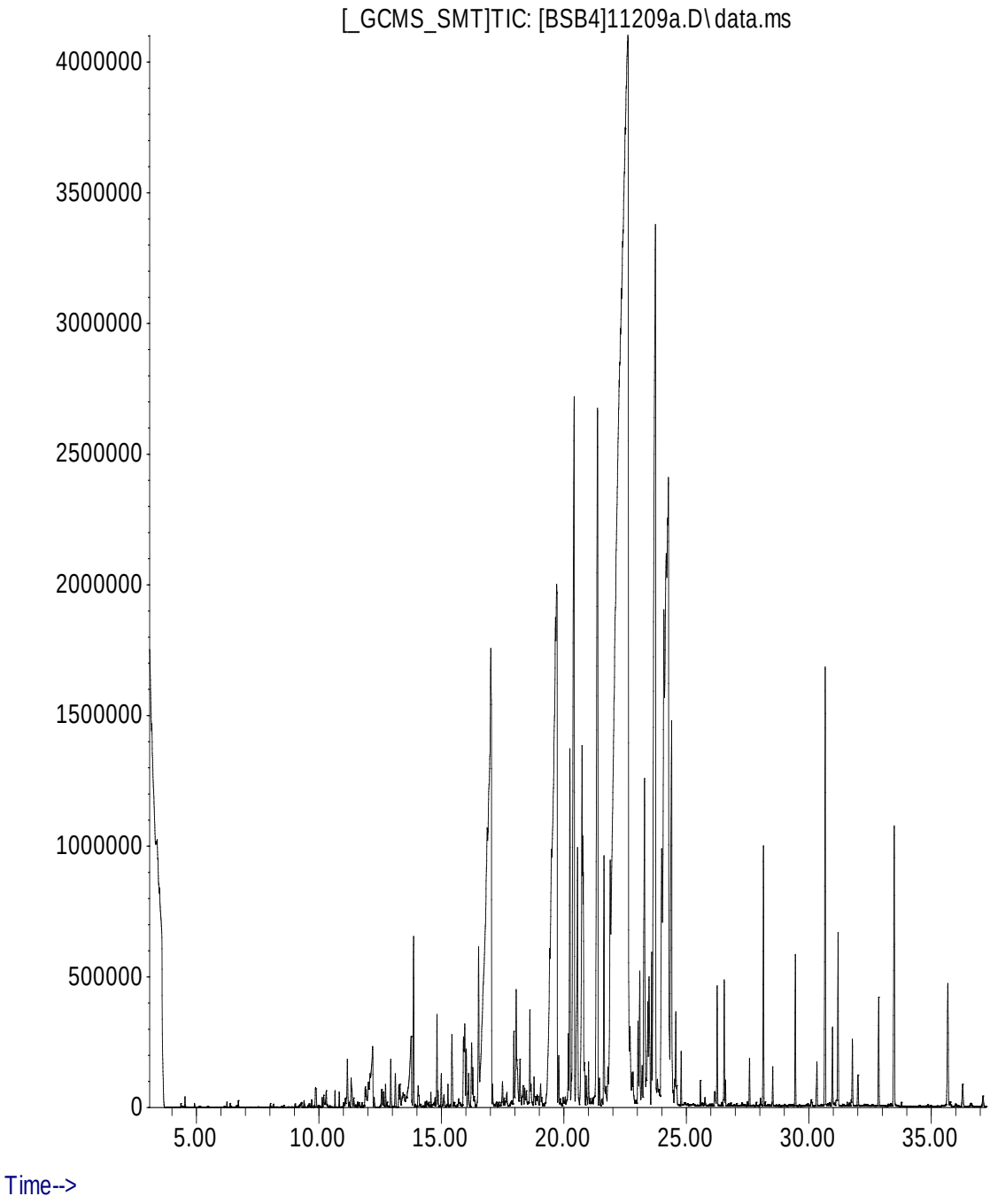


Figure 34: TIC of *Adansonia digitata* leaves essential oil collected after the 4th hr

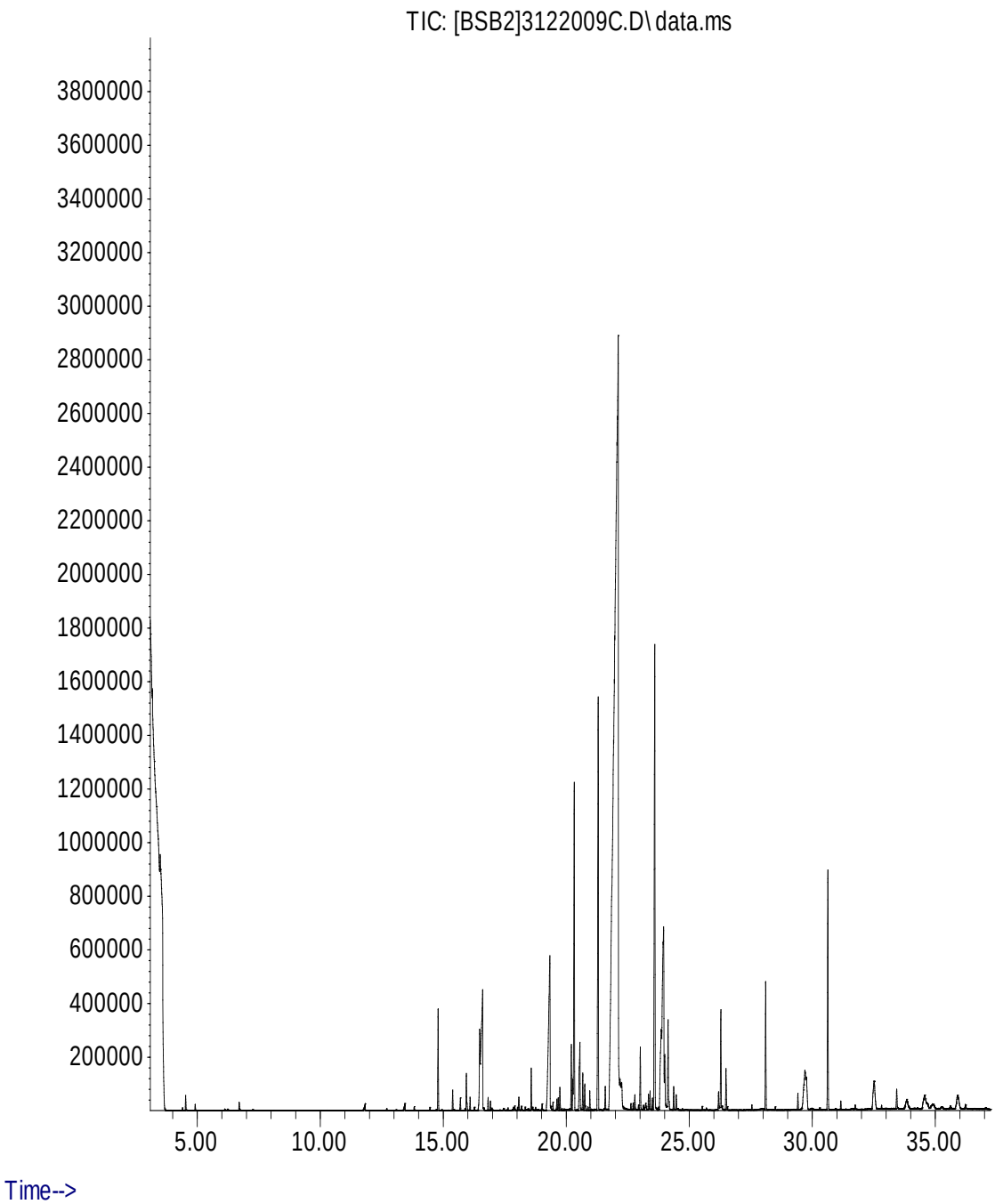


Figure 35: TIC of *Adansonia digitata* leaves essential oil collected after 4 hrs

Table 10: Compounds obtained from the GC-MS analyses of the essential oil samples from the leaves of *Adansonia digitata*

S/N	R/T (min)	Compounds	Formulae/Other names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs Cont.
1.	14.94	5,9-Undecadien-2-one,6,10-dimethyl-(E)-	$C_{13}H_{22}O$ Geranylacetone	Methylated secondary ketone	8.34	4.91	0.76	0.23	0.99
2.	16.87	Dodecanoic acid	$C_{12}H_{24}O_2$ Lauric acid	Saturated fatty acid (SFA)	7.34	9.37	8.38	7.59	3.11
3.	19.55	Tetradecanoic acid	$C_{14}H_{28}O_2$ Myristic acid	SFA	6.36	8.16	8.25	8.40	4.03
4.	20.25	Bicyclo[3,1.1.]heptane,2,6,6-trimethyl-(1 alpha.,2 beta., 5.alpha	$C_{10}H_{18}$ cis-Pinane	Hydrogenated Terpene	0.75			1.08	
5.	20.25	7-Methyl-1,6-octadiene	C_9H_{16}	Methylated Alkene			1.13		
6.	20.41	2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	Methylated secondary ketone	5.21	5.57	4.22	3.42	4.02
7.	20.55	Pentadecanoic acid	$C_{15}H_{30}O_2$	SFA		0.58			1.05
8.	20.56	Z-1,6-Tridecadiene	$C_{13}H_{24}$	Long chain alkene				0.96	
9.	20.73	Phthalic acid, isobutyl octyl ester	$C_{20}H_{30}O_4$	Ester		1.03	1.38		2.12
10.	21.38	5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl-(E,E)-	$C_{18}H_{30}O$ Farnesyl acetone	Methylated secondary ketone	5.16		0.41		5.15

SFA = Saturated fatty acid

Table 10 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Adansonia digitata*

S/N	R/T (min)	Compounds	Formulae/Other names	Classes	Percentage of Total				
					1st hr	**2nd hr**	**3rd hr**	**4th hr**	**4 hrs cont.**
11.	21.64	Isophytol	$C_{20}H_{40}O$	Diterpene alcohol	0.58		0.87	0.70	
12.	22.26	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ Palmitic acid	SFA	26.80	40.71	35.31	38.37	54.71
13.	23.13	Heptadecanoic acid	$C_{17}H_{34}O_2$	SFA	0.56	0.40	0.82	1.22	
14.	23.66	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	5.77	7.93	6.96	5.96	6.12
15.	24.06	9,12,15-Octadecatrienoic acid (Z,Z,Z)	$C_{18}H_{30}O_2$ Linolenic acid	Polyunsaturated fatty acid (PUFA)	5.17	7.06	3.25	10.23	7.28
16.	24.08	9,12,15-Octadecatrien-1-ol (Z,Z,Z)	$C_{18}H_{32}O$	Linolenyl alcohol			6.35		
17.	24.24	Octadecanoic acid	$C_{18}H_{36}O_2$	SFA	0.89	0.94	1.25	1.66	1.19
18.	28.10	Di-n-Octylphthalate	$C_{24}H_{38}O_4$	Ester		1.40			1.05
19.	30.63	Squalene	$C_{30}H_{50}$	Isoprenoid compound	1.55	1.39	1.55	2.24	2.69
TOTAL					**74.48**	**89.45**	**80.89**	**82.06**	**93.51**

SFA = Saturated fatty acid**;** **PUFA =** Polyunsaturated fatty acid

There are 9 constituent compounds identified by the GC-MS analysis of the essential oils from *Adansonia digitata* leaves which appeared in all the fractions (both hourly and single hour collections) obtained. Saturated fatty acids were 4 out of these constituents and they include dodecanoic acid (7.34, 9.37, 8.38, 7.59, 3.11% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 16.87 mins), tetradecanoic acid (6.36, 8.16, 8.25, 8.40, 4.03% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 19.55 mins), n-hexadecanoic acid (26.80, 40.71, 35.31, 38.37, 54.71, in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 22.26) and octadecanoic acid (0.89, 0.94, 1.25, 1.66, 1.19% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 24.24 mins). The other saturated fatty acids identified include pentadecanoic acid (0.58, 1.05% in 2^{nd}, 4 hrs respectively, R_T 20.55 mins) and heptadecanoic acid (0.56, 0.40. 0.82, 1.22% in 1st, 2^{nd}, 3^{rd}, 4^{th} hr respectively, RT 23.13 mins). Generally, saturated fatty acids had the highest percentage of total constituents obtained in each of the essential oil fractions (41.95, 60.16, 54.01, 57.24 and 64.09% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 4 hrs respectively). Some methylated ketones were identified in the fractions and they include 5, 9-undecadien-2-one, 6, 10-dimethyl-(E) - (8.336, 4.91, 0.76, 0.23, 0.99% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 14.94 mins), 2-pentadecanone, 6, 10, 14-trimethyl (5.21, 5.57, 4.22, 3.42, 4.02% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs, respectively, R_T 20.41 mins) and 5, 9, 13-pentadecatrien-2-one, 6, 10, 14-trimethyl-(E,E)- (5.16, 0.41, 5.15% in 1^{st}, 3^{rd}, 4 hrs, R_T 21.38 mins). Diterpene alcohols were also identified from the results and they include phytol (5.77, 7.93, 6.96, 5.96, 6.12% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 23.66 mins) and isophytol (0.58, 0.87, 0.70% in 1^{st}, 3^{rd}, 4^{th} hr respectively, R_T 21.64 mins). A long chain alcohol, 9, 12, 15-octadecatrien-1-ol (Z,Z,Z) [6.35% in 3^{rd} hr, R_T 24.08 mins) was present in the essential oil sample. Squalene, an isoprenoid (1.55, 1.39, 1.55, 2.24, 2.69% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 30.63 mins) and 9,12,15-octadecatrienoic acid (Z,Z,Z), polyunsaturated fatty acid (5.17, 7.06, 3.25, 10.23, 7.28% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 24.06 mins) were also identified in all the fractions of the essential oils. The

remaining compounds were two esters namely phthalic acid, isobutyl octyl ester (1.03, 1.38, 2.12% in 2^{nd}, 3^{rd}, 4 hrs respectively, R_T 20.73 mins), di-n-octylphthalate (1.40, 1.05% in 2^{nd}, 4 hrs respectively, R_T 28.10 mins), bicyclo [3.1.1.]heptane,2,6,6-trimethyl-(1 alpha.,2 beta., 5.alpha (0.75, 1.08% in 1^{st}, 4^{th} hr respectively, R_T 20.25 mins) which is a hydrogenated terpene and alkenes which include 7-methyl-1,6-octadiene (1.13% in 3^{rd} hr, R_T 20.25 mins) and Z-1,6-tridecadiene (0.96% in 4^{th} hr, R_T 20.56 mins).

4.2.2 GC-MS analyses of the essential oil samples from the leaves of *Calotropis procera*

The chromatograms obtained from the GC-MS analyses of the essential oil samples obtained from the 1^{st}, 2^{nd}, 4^{th} hourly fractions and 4 hrs single collection of *C. procera* are shown in Figures 36-39 and the retention times, names, formulae, classes and percentage abundances of various constituent compounds are presented in Table 12.

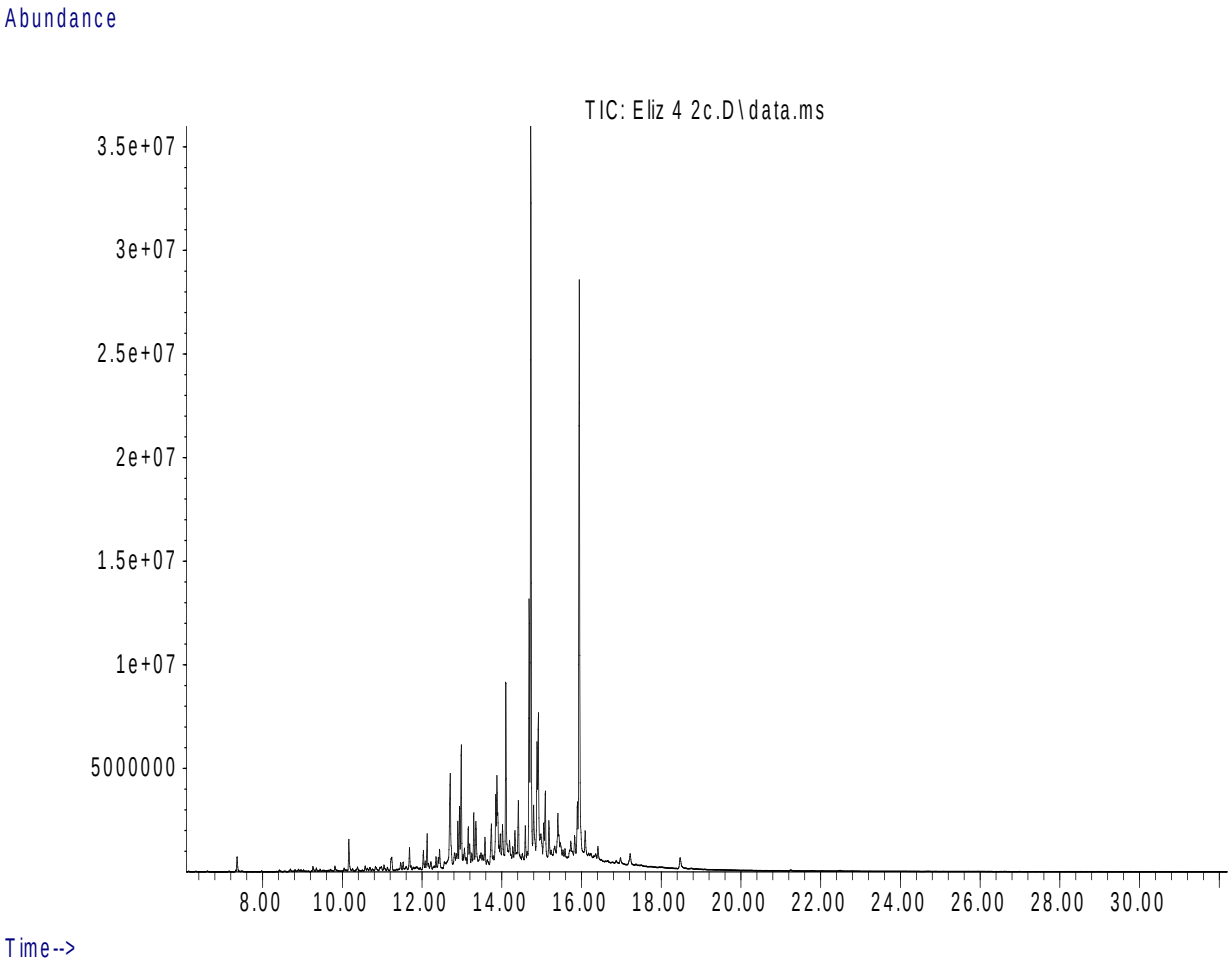


Figure 36: TIC of *Calotropis procera* leaves essential oil collected after 1st hr

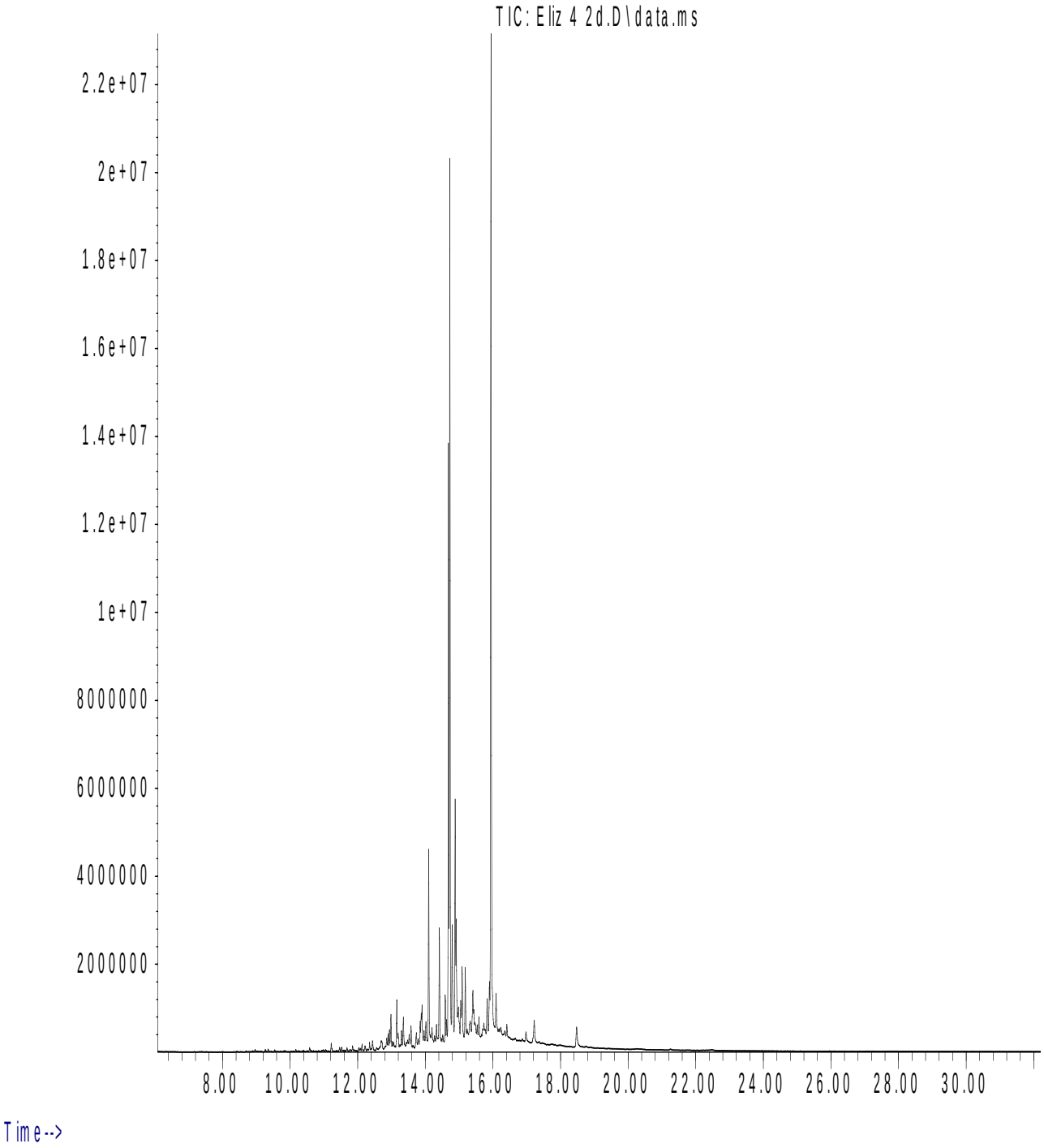


Figure 37: TIC of *Calotropis procera* leaves essential oil collected after 2nd hr

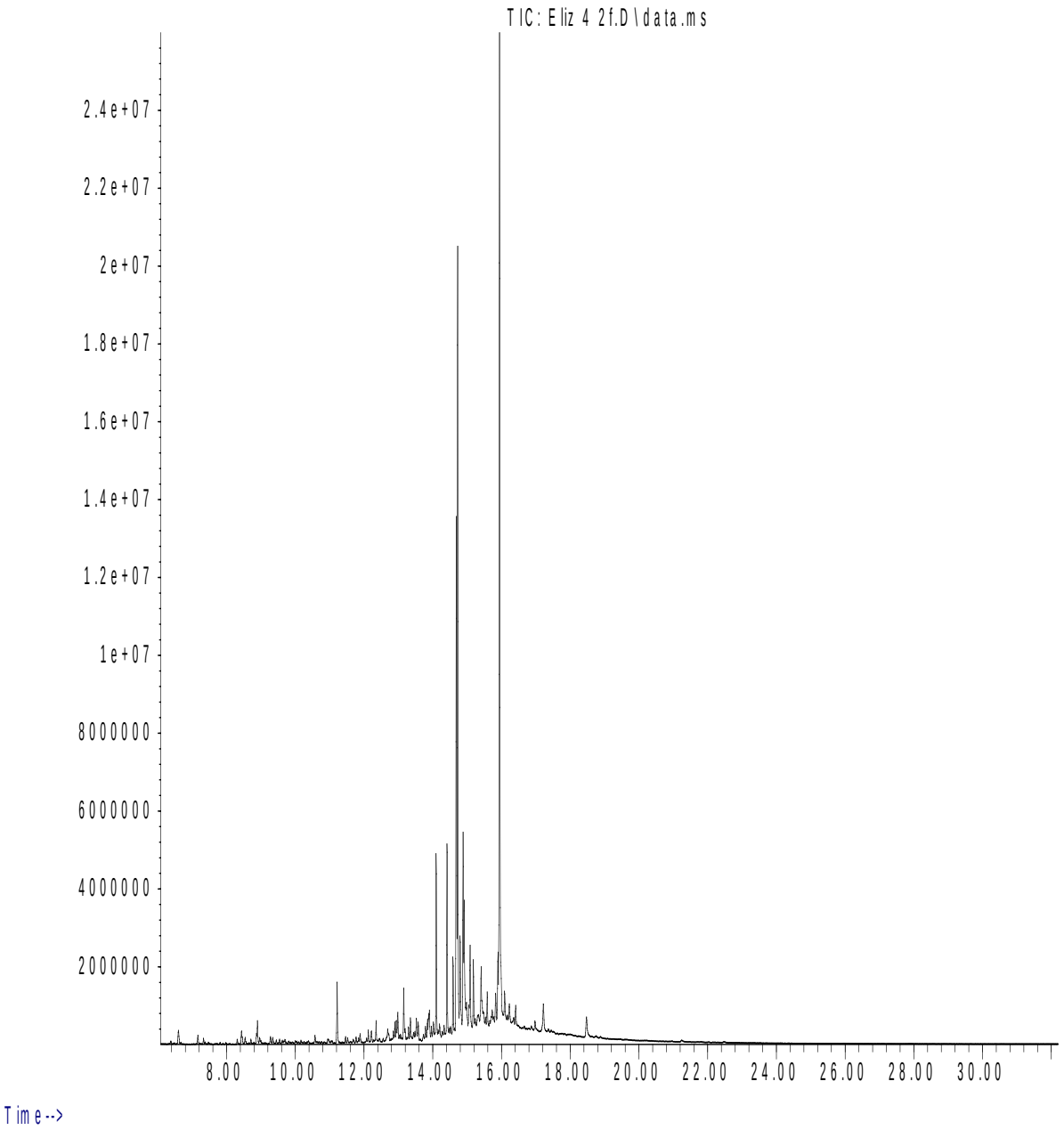


Figure 38: TIC of *Calotropis procera* leaves essential oil collected after 4th hr

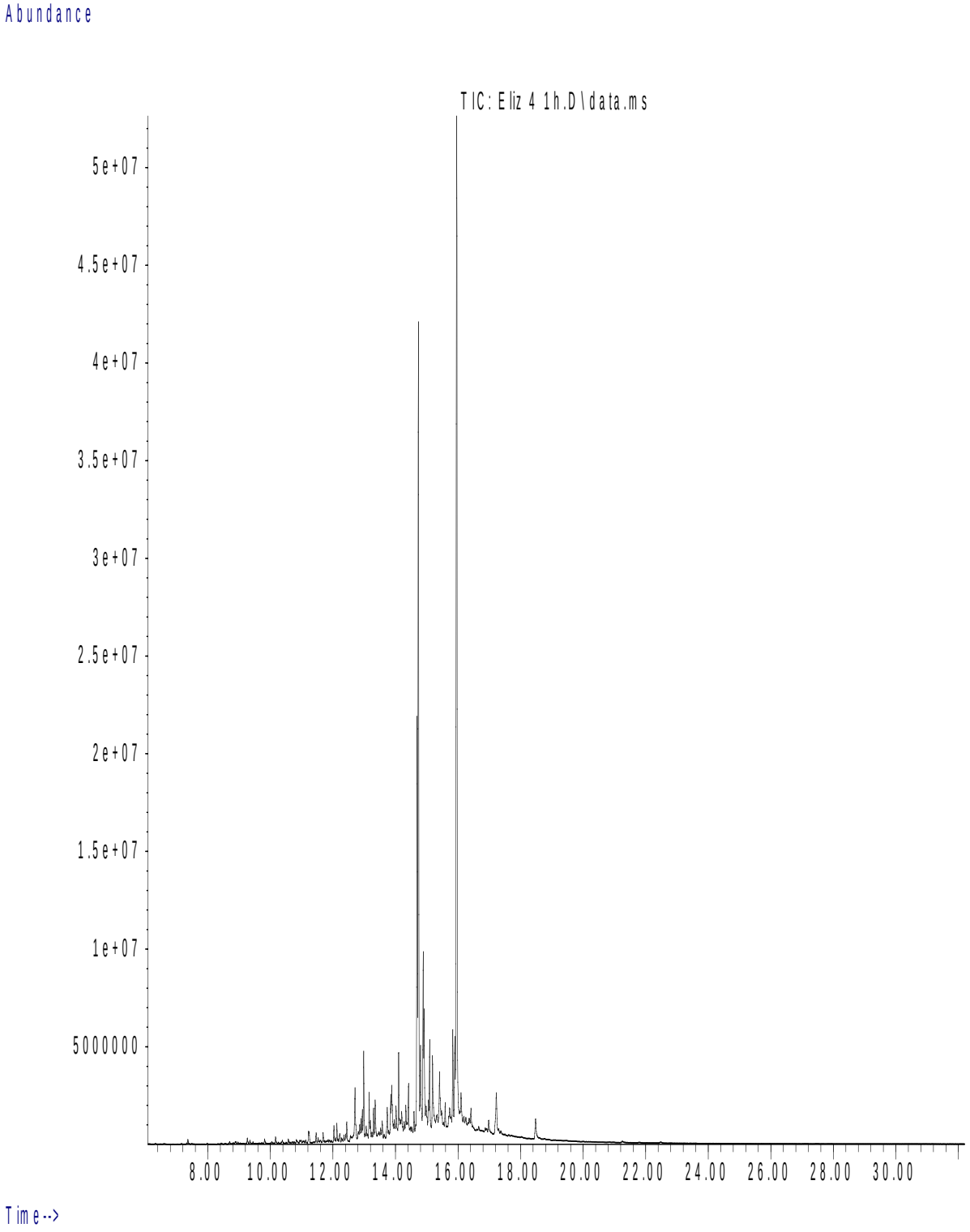


Figure 39: TIC of *Calotropis procera* leaves essential oil collected after 4 hrs

Table 11: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Calotropis procera*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
1.	12.71	2-Butanone,4-(2,6,6-trimethyl—cyclohexen-1-yl)-	$C_{13}H_{22}O$	Secondary ketone	3.18				1.57
2.	12.94	2-Tridecanone	$C_{13}H_{26}O$	Secondary ketone	1.08				
3.	12.98	3-Buten-2-one, 4(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-	$C_{13}H_{22}O$	Secondary ketone	2.43	0.74	0.43	0.61	1.48
4.	13.89	9,12-Octadecadienoyl chloride,(Z,Z)-	$C_{18}H_{31}ClO$ Linoleoyl chloride	Fatty acid chloride					1.53
5.	13.90	9, 17-Octadecadienal, (Z, Z)-	$C_{18}H_{32}O$	Aldehyde	2.84	1.49			
6.	14.10	Tetradecanal	$C_{14}H_{28}O$ Myristaldehyde	Aldehyde			3.10	3.38	
7.	14.10	Hexadecanal	$C_{16}H_{32}O$	Aldehyde	4.14	1.01	2.83	3.60	1.71
8.	14.69	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ Isomer of phytol	Methylated alcohol	4.74	10.28	12.62	8.46	6.79
9.	14.73	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	Methylated secondary ketone	14.44	16.63	14.99	13.83	15.31
10.	14.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ Isomer of phytol	Methylated alcohol	4.62	7.96	10.59	6.38	5.60
11.	14.91	9 - Nonadecene	$C_{19}H_{38}$	Long chain alkene		2.77			

Table 11 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Calotropis procera*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4hrs cont.
12.	14.92	Z-5-Nonadecene	$C_{19}H_{38}$	Long chain Alkene	3.25				2.27
13.	14.92	1-Nonadecene	$C_{19}H_{38}$	Long chain Alkene				3.13	
14.	15.09	5, 9, 13-Pentadecatriene-2-one, 6, 10, 14-trimethyl (E, E) -	$C_{18}H_{30}O$	Methylated secondary ketone		1.97	2.14	2.70	1.88
15.	15.18	Isophytol	$C_{20}H_{40}O$	Diterpene alcohol		1.70	1.81	1.62	
16.	15.41	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	Methylated alcohol	2.08	1.54	1.91		1.95
17.	15.89	1-Docosanol	$C_{22}H_{46}O$	Long chain alcohol		1.29		2.28	
18.	15.97	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	14.44	22.41	30.97	21.81	25.19
19.	17.23	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	Lactone			1.06		1.34
TOTAL					**57.24**	**69.79**	**82.45**	**67.80**	**66.62**

The compounds present in the first hour fraction of leaf essential oil of *C. procera* include phytol (14.44, 22.41, 30.97, 21.81, 25.19% in 1st, 2nd, 3rd, 4th and 4 hrs repectively, R_T 15.97 mins), 2-pentadecanone, 6, 10, 14-trimethyl- (14.44, 16.63, 14.99, 13.83, 15.31% in 1st, 2nd, 3rd, 4th and 4 hrs, respectively, R_T 14.73 mins), 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer (9.36, 18.24, 23.21, 14.84, 12.40% in 1st, 2nd, 3rd, 4th and 4 hrs respectively, R_T 14.69, 14.80 mins), 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (3.18, 1.57% in 1st and 4 hrs respectively, R_T 12.71 mins), 3-buten-2-one,4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (2.43, 0.74, 0.43, 0.61, 1.48% in 1st, 2nd, 3rd, 4th and 4 hrs repectively, R_T 12.98 mins), 9, 17-octadecadienal (2.84, 1.49% in 1st and 2nd hr, R_T 13.90 mins), hexadecanal (4.14, 1.01, 2.83, 3.60, 1.71% in 1st, 2nd, 3rd, 4th and 4 hrs respectively, R_T 14.10 mins), Z-5-nonadecene (3.25, 2.27%, in 1st and 4 hrs respectively, R_T 14.92 mins) and 2-methyl-1-hexadecanol (2.08, 1.54, 1.91, 1.95% in 1st, 2nd, 3rd and 4 hrs respectively, R_T 15.41 mins).The second hour fraction of the essential oils contains the compounds identified in the first hour except 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl and Z-5- nonadecene. However, in addition it contains 5, 9, 13-pentadecatriene-2-one, 6, 10, 14-trimethyl (E, E) [1.97, 2.14, 2.70, 1.88% in 2nd, 3rd, 4th, and 4 hrs respectively, R_T 15.09 mins), 9-nonadecene, isophytol (1.70, 1.81, 1.62% in 2nd, 3rd and 4th hr respectively, R_T 15.18 mins) and I-docosanol (1.29, 2.28% in 2nd and 4th hr respectively, R_T 15.89 mins). The constituents of the third hourly fraction were essentially those contained in the second hourly fraction with the exception of 9, 17-octadecadienal and 1-docosanol. However, in addition, tetradecanal (3.10, 3.38% in 3rd and 4th hr respectively, R_T 14.10 mins) and 4, 8, 12, 16-tetramethylheptadecan-4-olide (1.06, 1.34% in 3rd and 4 hrs respectively, R_T 17.23 mins) were present. The constituents in the fourth hourly fraction were almost the same as those in the third except 4, 8, 12, 16-tetramethylheptadecan-4-olide but with the addition of 1-docosanol. The major constituents of the essential oil collected over the entire period of 4 hrs were the same as those in the hourly extraction and were phytol, 6, 10,

14-trimethyl-2-pentadecanone, 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer. The minor constituents identified were 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl, 3-buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl, hexadecanal, Z-5-nonadecene, 5, 9, 13-pentadecatriene-2-one-6, 10, 14-trimethyl (E, E), 2-methyl-1-hexadecanol, 4, 8, 12, 16-tetramethylheptadecan-4-olide in addition to 9,12-ctadecadienoyl chloride (Z,Z)- (1.53% in 4 hr, R_T 13.89 mins).

4.2.3 GC-MS Analyses of the essential oil samples from the leaves of *Coix lacryma-jobi*

The chromatograms obtained from the GC-MS analyses of the essential oil samples from the 1st, 2nd hourly fractions and 4 hrs single collection of *C. lacryma-jobi* are shown in Figures 40 - 42 and the retention times, names, formulae, classes and percentage abundances of various constituent compounds are presented in Table 12.

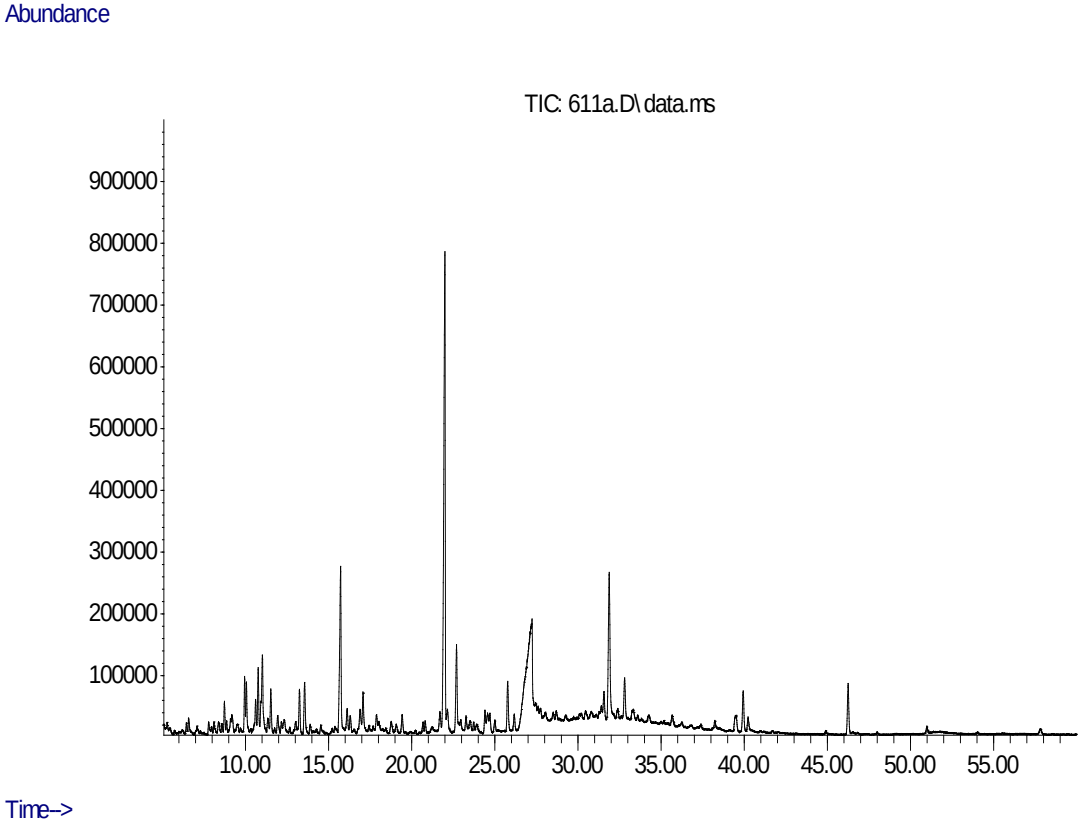


Figure 40: TIC of *Coix lacryma-jobi* leaves essential oil collected after 1st hr

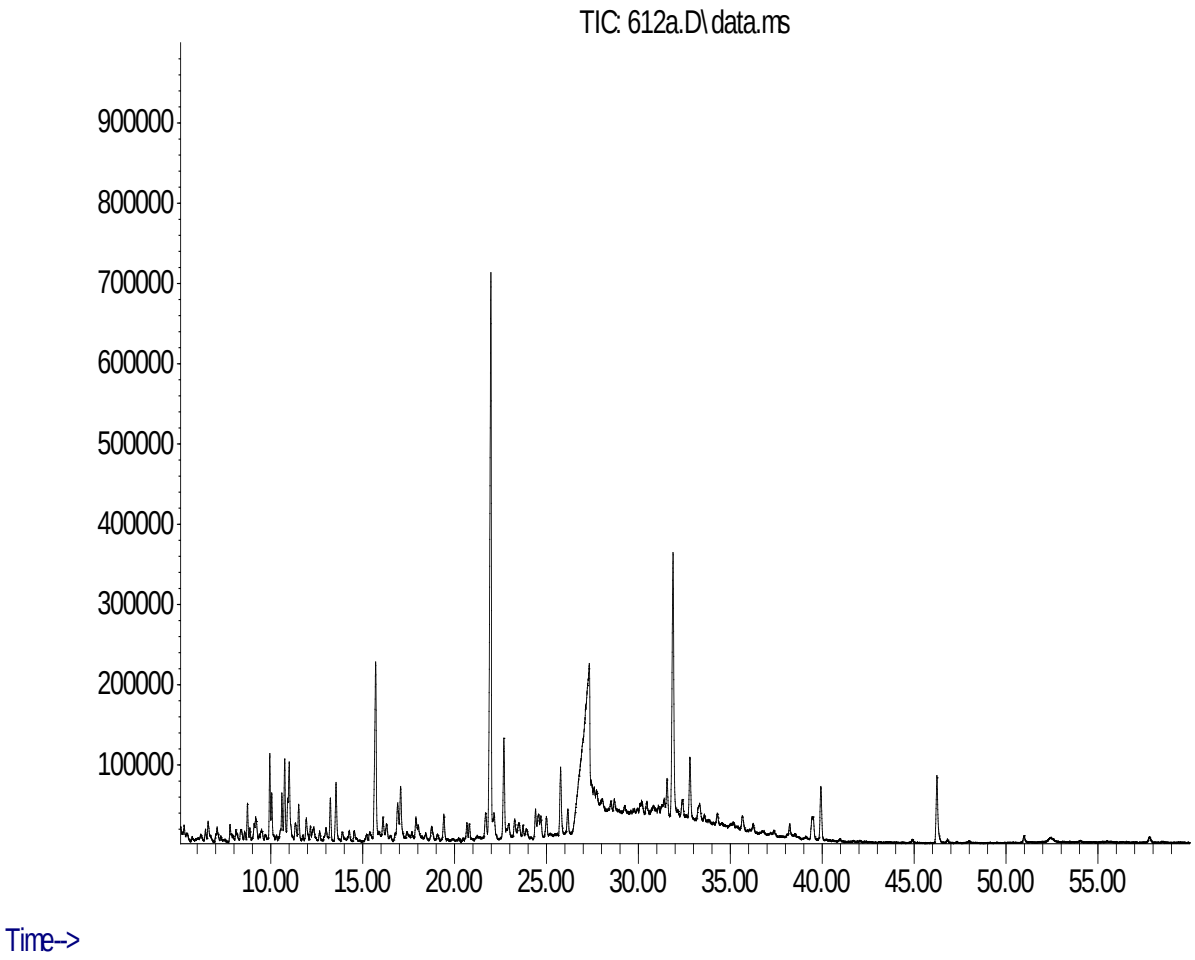


Figure 41: TIC of *Coix lacryma-jobi* leaves essential oil collected after 2nd hr

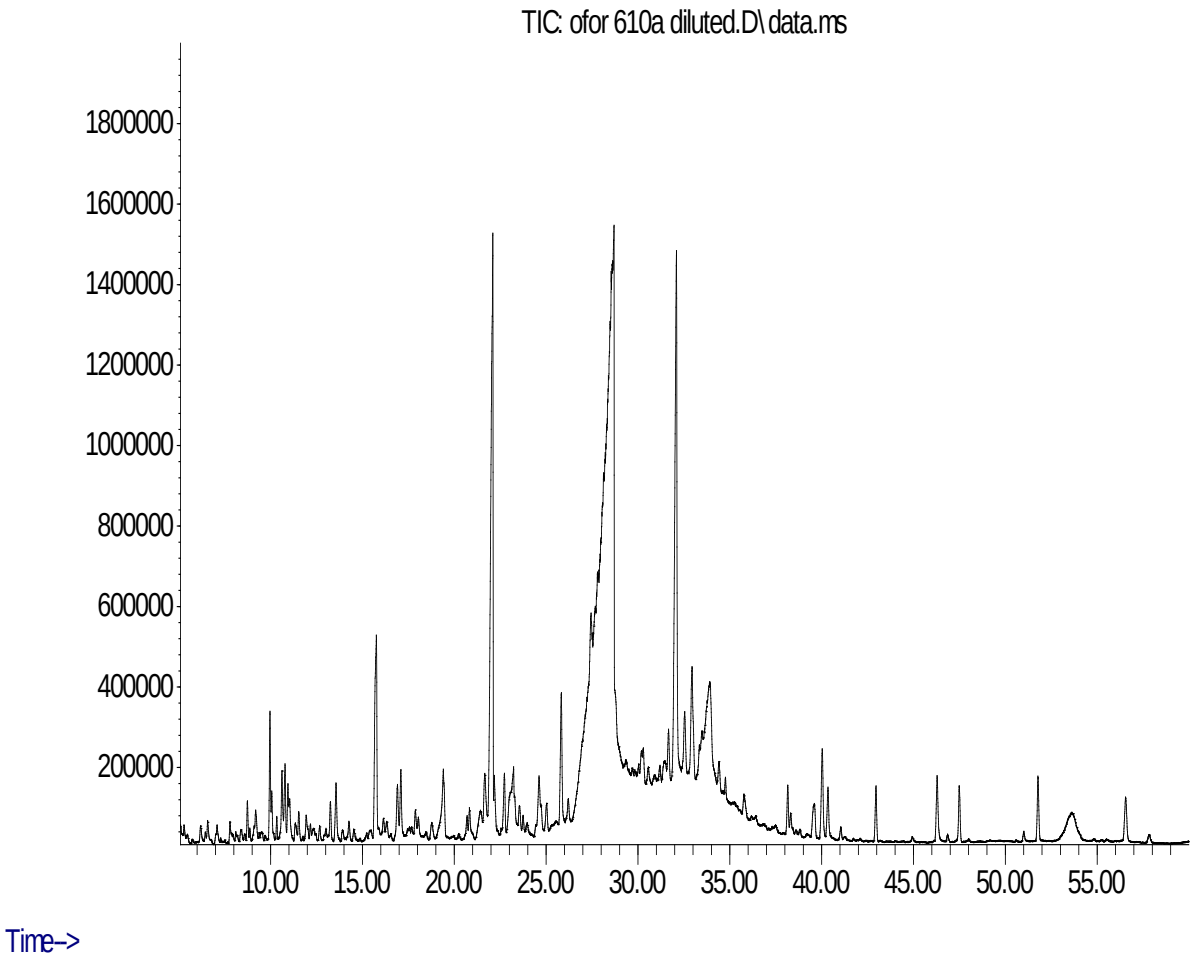


Figure 42: TIC of *Coix lacryma-jobi* leaves essential oil collected after 4 hrs

Table 12: **Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Coix lacryma-jobi***

S/n	R/T (min)	Compounds	Formulae/ Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
1.	9.97	3-Buten-2-one, 4-(2,6,6-trimethyl- 1- cyclohexen-1-yl)-, (E)-	$C_{13}H_{20}O$ (E)-β-Ionone	Methylated secondary ketone		2.09			0.79
2.	10.77	Cyclohexanecarboxylic acid, 4-formylphenyl ester	$C_{14}H_{16}O_3$	Ester	2.25				
3	11.01	2-Methyl-7-exo-vinylbicyclo[4.2.0]oct-1(2)-ene	$C_{11}H_{16}$	Alkene		2.67			
4	11.02	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	Methylated ketone	3.25				
5.	11.52	(+)-Epi-bicyclosesqui phellandrene	$C_{15}H_{24}$	Sesquiterpene		1.12			
6	15.73	N.I.			8.26	6.70			
7.	17.06	1,15-Pentadecanediol	$C_{15}H_{32}O_2$	Fatty alcohol		2.09			

Table 12 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Coix lacryma-jobi*

S/n	R/T (min)	Compounds	Formulae/ Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
8.	17.07	1,12-Tridecadiene	$C_{13}H_{24}$	Lon chain alkene	1.93				
9.	22.10	2-Pentadecanone, 6,10,14-trimethyl -	$C_{18}H_{36}O$	Methylated secondary ketone	24.00	20.35	27.28	15.78	10.49
10.	22.70	N.I.			3.45	3.28			
11.	23.23	Pentadecanoic acid	$C_{15}H_{30}O_2$	SFA			2.35		0.54
12.	24.62	5,9,13-Pentadecatrien-2-one, 6,10, 14-trimethyl-, (E,E)-	$C_{18}H_{30}O$ Farnesyl acetone	Methylated secondary ketone					1.28
13.	24.99	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ Methyl palmitate	Fatty acid ester				1.30	
14.	25.77	N.I.				2.52			

SFA = Saturated fatty acid**;** **N.I. =** Not Identified

Table 12 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Coix lacryma-jobi*

S/n	R/T (min)	Compounds	Formulae/ Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
15.	25.83	Isophytol	$C_{20}H_{40}O$	Acyclic Diterpene alcohol			4.52	3.95	1.64
16.	26.99-28.70	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ Palmitic acid	SFA	18.33	20.59	23.36	38.67	40.35
17.	31.89	N.I.				10.89			
18.	32.09	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	8.03		8.26	20.85	8.61
19.	32.80	N.I.				2.49			
20.	33.91	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$ Linoleic acid	PUFA					4.66
21.	40.03	4,8,12,16-Tetra methyl heptadecan-4-olide	$C_{21}H_{40}O_2$	Methylated ketone	2.02	2.17	3.65	2.45	1.26
22.	40.35	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$ Oleamide	Fatty acid amide					0.64
23.	46.25	N.I.			2.43	2.98			
TOTAL					**73.95**	**79.94**	**69.42**	**83.00**	**70.26**

SFA = Saturated fatty acid**;** **PUFA =** Polyunsaturated fatty acid; **N.I**. = Not Identified

The compounds with the highest percentage of total and which were identified in all the fractions of essential oils of *C. lacryma-jobi* were 2-pentadecanone, 6,10,14-trimethyl – (24.00, 20.35, 27.28, 15.78, and 10.49% in 1st, 2nd, 3rd, 4th and 4 hrs respectively, R_T 22.10 mins), n-hexadecanoic acid, a saturated fatty acid (18.33, 20.59, 23.36, 38.67, 40.35% in 1st, 2nd, 3rd, 4th, 4 hrs respectively, R_T 26.99 - 28.70 mins) and 4,8,12,16-tetra methyl heptadecan-4-olide (2.02, 2.17, 3.65, 2.45, 1.26% in 1st, 2nd, 3rd, 4th, 4 hrs respectively, R_T 40.03 mins). These major compounds add up to 44.35, 43.11, 54.29, 56.90 and 52.10% for 1st, 2nd, 3rd, 4th and 4 hrs respectively out of the total constituent compounds. Phytol (8.03, 8.26, 20.85, 8.61% in 1st, 3rd, 4th, 4 hrs respectively, R_T 32.09 mins), a diterpene alcohol was also identified in high yield though it was absent at the 2nd hr fraction. The remaining compounds were 3-Buten-2-one, 4-(2,6,6-trimethyl- 1- cyclohexen-1-yl)-, (E)- (2.09, and 0.79% in 2nd, 4 hrs respectively, R_T 9.97 mins), cyclohexanecarboxylic acid, 4-formylphenyl ester (2.25% in 1st hr, R_T 9.97 mins), (+)-Epi-bicyclo sesquiphellandrene (1.12% in 2nd hr, R_T 11.52 mins), 1,15-pentadecanediol (2.09% in 2nd hr, R_T 17.07 mins), pentadecanoic acid (2.35, 0.54% in 3rd, 4 hrs respectively, R_T 23.23 mins), 5,9,13-pentadecatrien-2-one, 6, 10, 14-trimethyl-, (E,E)- (1.28% in 4 hr, R_T 24.62 mins), hexadecanoic acid, methyl ester (1.30% in 4th hr, R_T 24.99 mins), isophytol (4.52, 3.95, 1.64% in 3rd, 4th, 4 hrs respectively, R_T 25.83 mins), 9,12-octadecadienoic acid (Z,Z)-(4.66% in 4 hr, R_T 33.91) and 9-octadecenamide, (Z)- (0.64% in 4 hr, R_T 40.35 %).

4.2.4 GC-MS Analyses of the Essential oil Samples from the Leaves of *Datura metel*

The chromatograms obtained from the GC-MS analyses of the essential oil samples from the 1st, 2nd, 3rd hourly fractions and 4 hrs single collection of *Datura metel* are shown in Figures 43 - 46 and the retention times, names, formulae, classes and percentage abundances of various constituent compounds are presented in Table 13.

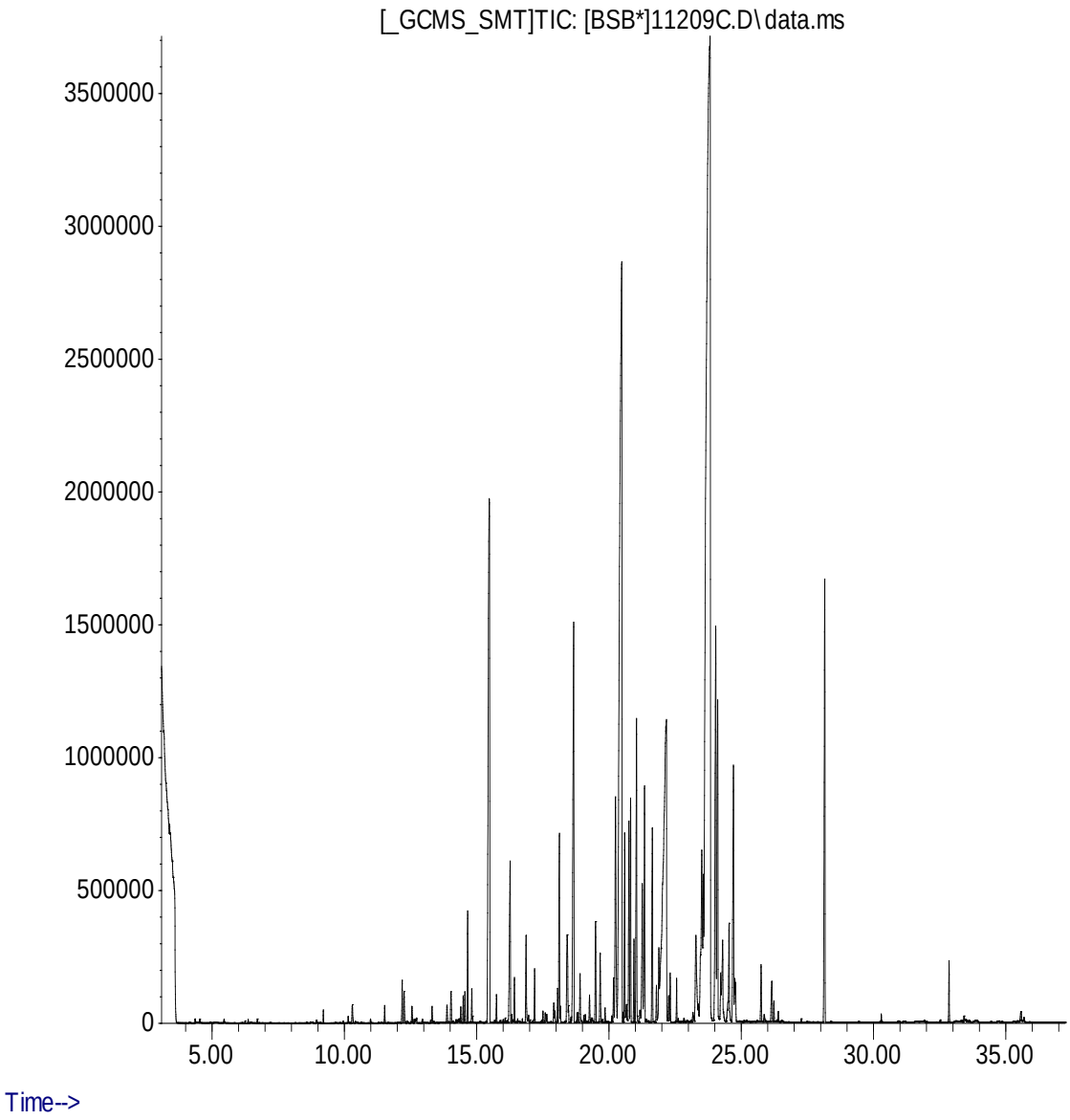


Figure 43: TIC of *Datura metel* leaves essential oil collected after 1st hr

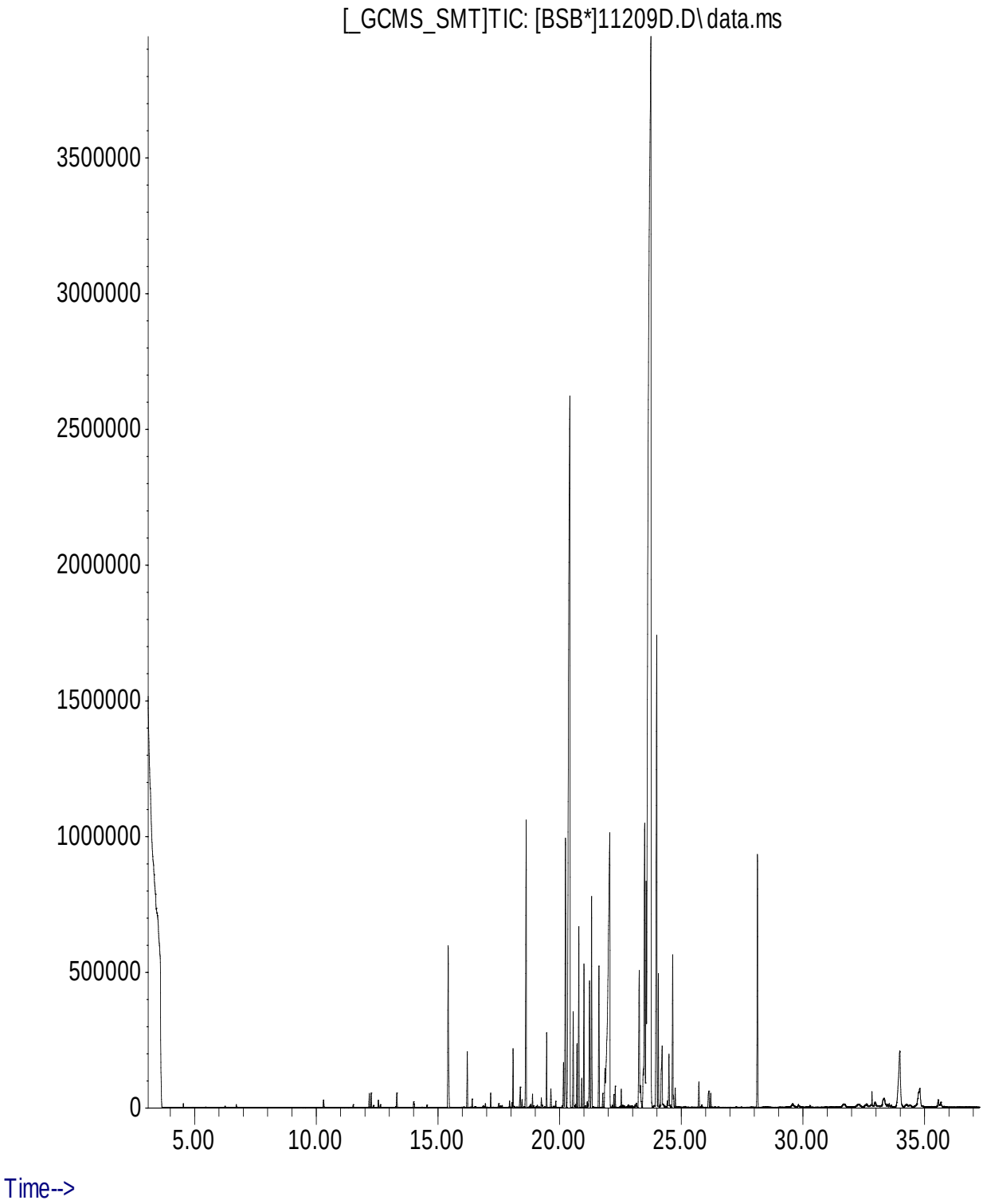


Figure 44: TIC of *Datura metel* leaves essential oil collected after 2nd hr

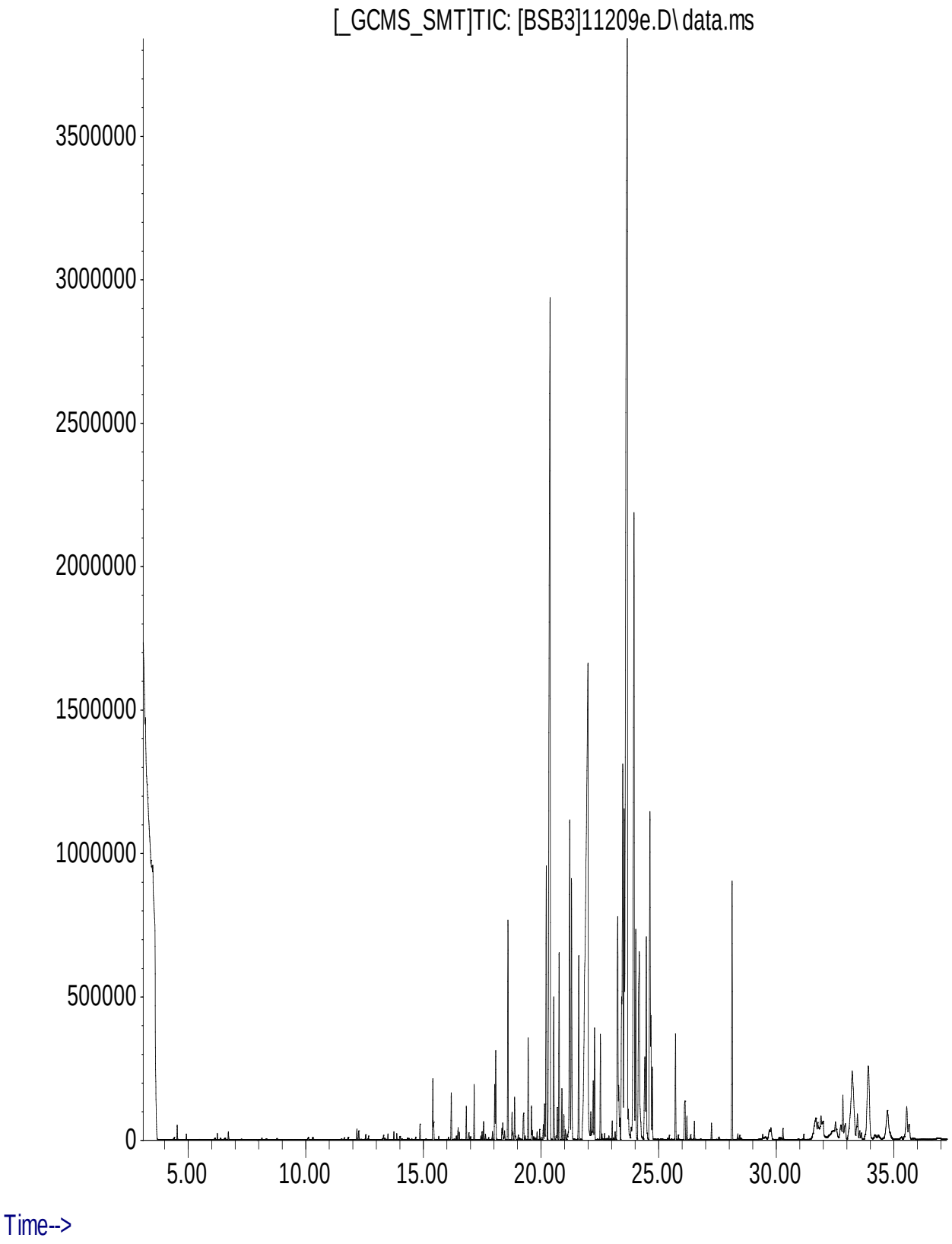


Figure 45: TIC of *Datura metel* leaves essential oil collected after 3rd hr

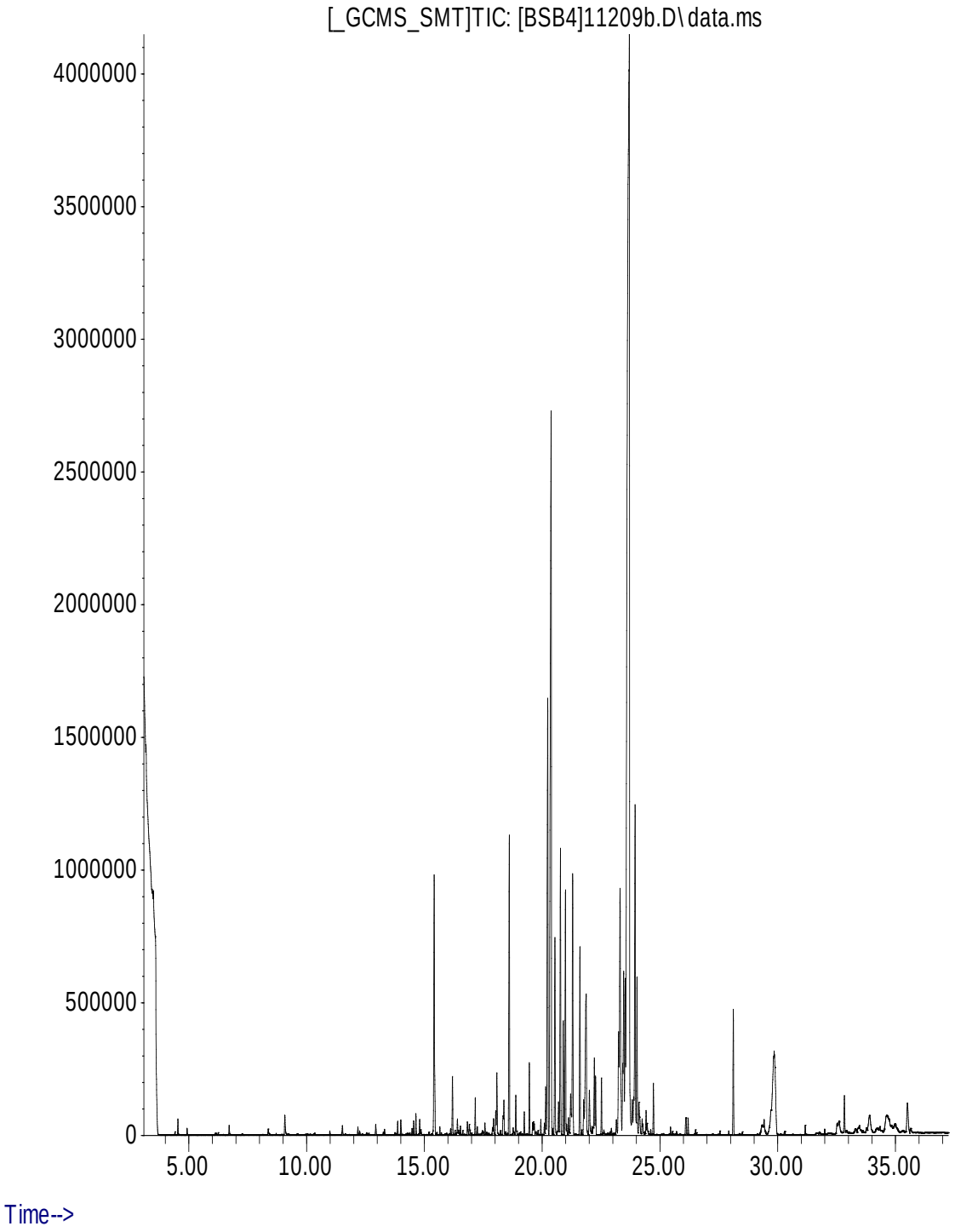


Figure 46: TIC of *Datura metel* leaves essential oil collected after 4 hrs

Table 13: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Datura metel*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
1.	15.42	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{20}O$ β-Ionone	Secondary ketone					2.60
2.	16.26	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	Ketone	1.30	0.42	0.32	0.24	0.52
3.	18.60	trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	Unsaturated fatty acid alcohol			1.42		
4.	18.60	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	Unsaturated fatty acid alcohol					2.63
5.	18.61	18-Nona decen-1-ol	$C_{19}H_{38}O$	Unsaturated fatty acid alcohol				1.53	
6.	18.62	Trichloroacetic acid, undec-10-enyl ester	$C_{13}H_{21}Cl_3O_2$	Ester		2.58			
7.	20.23	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$ Pinane	Hydrogenated terpene					5.11

Table 13 contd: **Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Datura metel***

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
8.	20.47	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	Methylated secondary ketone	14.16	14.90	11.21	10.93	10.66
9.	20.54	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ Isomer of phytol	Methylated alcohol			1.22		1.78
10	20.75	N.I			2.13				
11.	20.99	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$ Linolenic acid	PUFA	1.78				2.20
12.	21.22	6-Methyl-2-tridecanone	$C_{14}H_{28}O$	Secondary ketone			2.80		
13.	21.60	Isophytol	$C_{20}H_{40}O$	Acyclic diterpene alcohol					1.63
14.	21.62	3-Buten-2-ol, 1-bromo-2-methyl-	C_5H_9BrO	Brominated alcohol				1.55	
15.	22.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ Palmitic acid	SFA	7.87	7.70	13.07	15.63	2.64
16.	23.26	Valeric acid, 3-pentadecyl ester	$C_{20}H_{40}O_2$	Ester	2.73				
17.	23.27	Valeric acid, 4-pentadecyl ester	$C_{20}H_{40}O_2$	Ester		3.01			

SFA = Saturated fatty acid**;** **PUFA =** Polyunsaturated fatty acid; N.I. = Not Identified

Table 13 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Datura metel*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
18.	23.46	Eicosane, 2,4-dimethyl	$C_{22}H_{46}$	Methylated alkane			3.73		
19.	23.47	2-Octyn-1-ol, 7-[(tetrahydro-2H-pyran-2-yl)oxy]-	$C_{13}H_{22}O_3$	Alcohol	4.43				
20.	23.49	Tetrahydropyran 12-tetradecyn-1-ol ether	$C_{19}H_{34}O_2$	Ether		5.68			
21.	23.74	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	31.33	42.35	22.93	36.80	33.12
22.	23.94	Di(cyclopentanonyl-2)methane	$C_{11}H_{16}O_2$	Alkane					3.75
23.	23.95	4,4,6-Trimethyl-cyclohex-2-en-1-ol	$C_9H_{16}O$	Methylated unsaturated alcohol			6.76		
24.	23.98	Glyodin	$C_{22}H_{44}N_2O_2$	Ester				4.96	
25.	24.03	N.I.			3.20				
26.	24.11	N.I.			2.26				
27.	24.17	Octadecanoic acid	$C_{18}H_{36}O_2$ Stearic acid	SFA			2.83	1.41	
28.	32.42	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	Ester				1.22	
TOTAL					**71.19**	**76.64**	**66.29**	**74.27**	**66.64**

SFA = Saturated fatty acid**;** **N.I**. = Not Identified

The four major compounds identified by the GC-MS analysis of the different fractions of essential oils obtained from *D. metel* leaves were 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (1.30, 0.42, 0.32, 0.24, 0.52% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 16.820 mins), 2-pentadecanone, 6,10,14-trimethyl, a methylated ketone (14.16, 14.90, 11.21, 10.93, 10.66% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 20.471 mins), n-hexadecanoic acid , a saturated fatty acid (7.87, 7.70, 13.07, 15.63, 2.64% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 22.159 mins) and phytol (42.35, 22.93, 36.80, 33.12% in 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 23.740 mins). These sum up to 23.33, 65.37, 47.53, 66.60 and 46.94% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively of the total constituent compounds in each of the fractions. Many fatty acid alcohols were identified in the essential oil fractions and they include trans-2-dodecen-1-ol (1.42% in 3^{rd} hr, R_T 18.60 mins), E-2-tetradecen-1-ol (2.63% in 4 hrs, R_T 18.60 mins), 18-nona decen-1-ol (1.53% in 4^{th} hr, R_T 18.61 mins) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.22, 1.78% in 3^{rd}, 4 hrs respectively, R_T 20.54 mins). Other alcohols include isophytol (1.63% in 4 hrs, R_T 21.60 mins), 3-buten-2-ol, 1-bromo-2-methyl- (1.55% in 4^{th} hr, R_T 21.62 mins), 2-octyn-1-ol, 7-[(tetrahydro-2H-pyran-2-yl)oxy]- (4.43% in 1^{st} hr, R_T 23.47 mins), tetrahydropyran 12-tetradecyn-1-ol ether (5.68% in 2^{nd} hr, R_T 23.49 mins), 4,4,6-trimethyl-cyclohex-2-en-1-ol (6.76% in 3^{rd} hr, R_T 23.95 mins). Methylated secondary ketones identified in the essential oil samples include 3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (2.60% in 4 hrs, R_T 15.42 mins), 3-buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- (6.10% in 1^{st} hr, R_T 15.47 mins) and 6-methyl-2-tridecanone (2.80% in 3^{rd} hr, R_T 21.22 mins). Other minor compounds identified in the essential oil samples were esters which include trichloroacetic acid, undec-10-enyl ester (2.58% in 2^{nd} hr, R_T 18.62 mins), valeric acid, 3-pentadecyl ester (2.73% in 1^{st} hr, R_T 23.26 mins), valeric acid, 4-pentadecyl ester (3.01% in 2^{nd} hr, 23.27 mins), glyodin (4.96% in 4^{th} hr, R_T 23.98 mins) and dodecanoic acid, 1,2,3-propanetriyl ester, a fatty acid ester (1.22% in 4^{th} hr, R_T 32.42 mins); methylated

alkanes which include bicyclo[3.1.1]heptane, 2,6,6-trimethyl- (5.11% in 4 hrs, RT 20.23 mins) and eicosane, 2,4-dimethyl (3.73% in 3rd hr, R_T 23.46 mins) and di(cyclopentanonyl-2) methane (3.75% in 4 hrs, R_T 23.94 mins).

4.2.5 GC-MS Analyses of the Essential oil Samples from the Leaves of *Deinbollia pinnata*

The chromatograms obtained from the GC-MS analyses of the essential oil samples from the1st hourly fraction and 4 hrs single collection of *Deinbollia pinnata* are shown in Figures 47 and 48 and the retention times, names, formulae, classes and percentage abundances of the various constituent compounds are presented in Table 14.

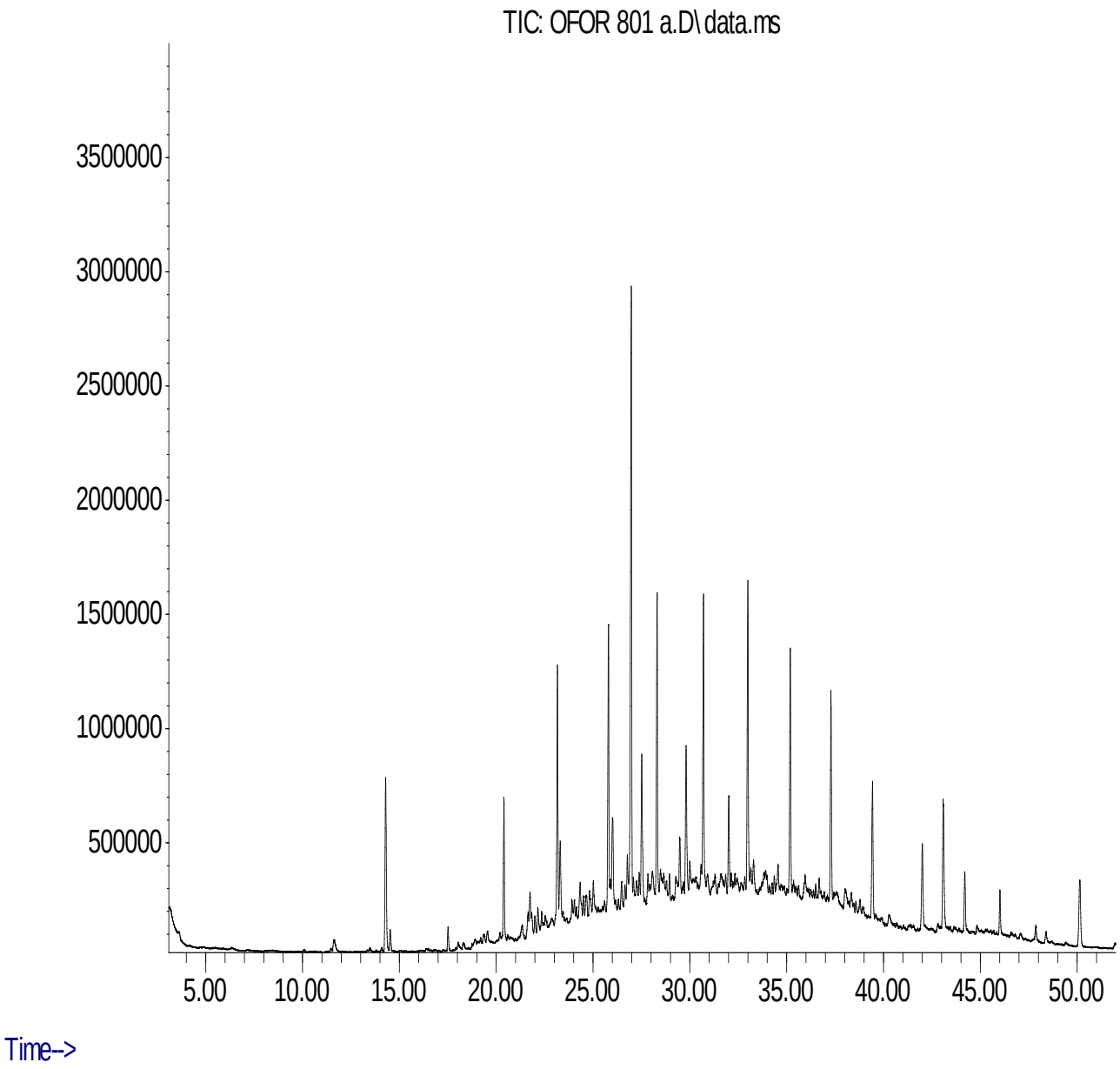


Figure 47: TIC of *Deinbollia pinnata* leaves essential oil collected after 1st hr

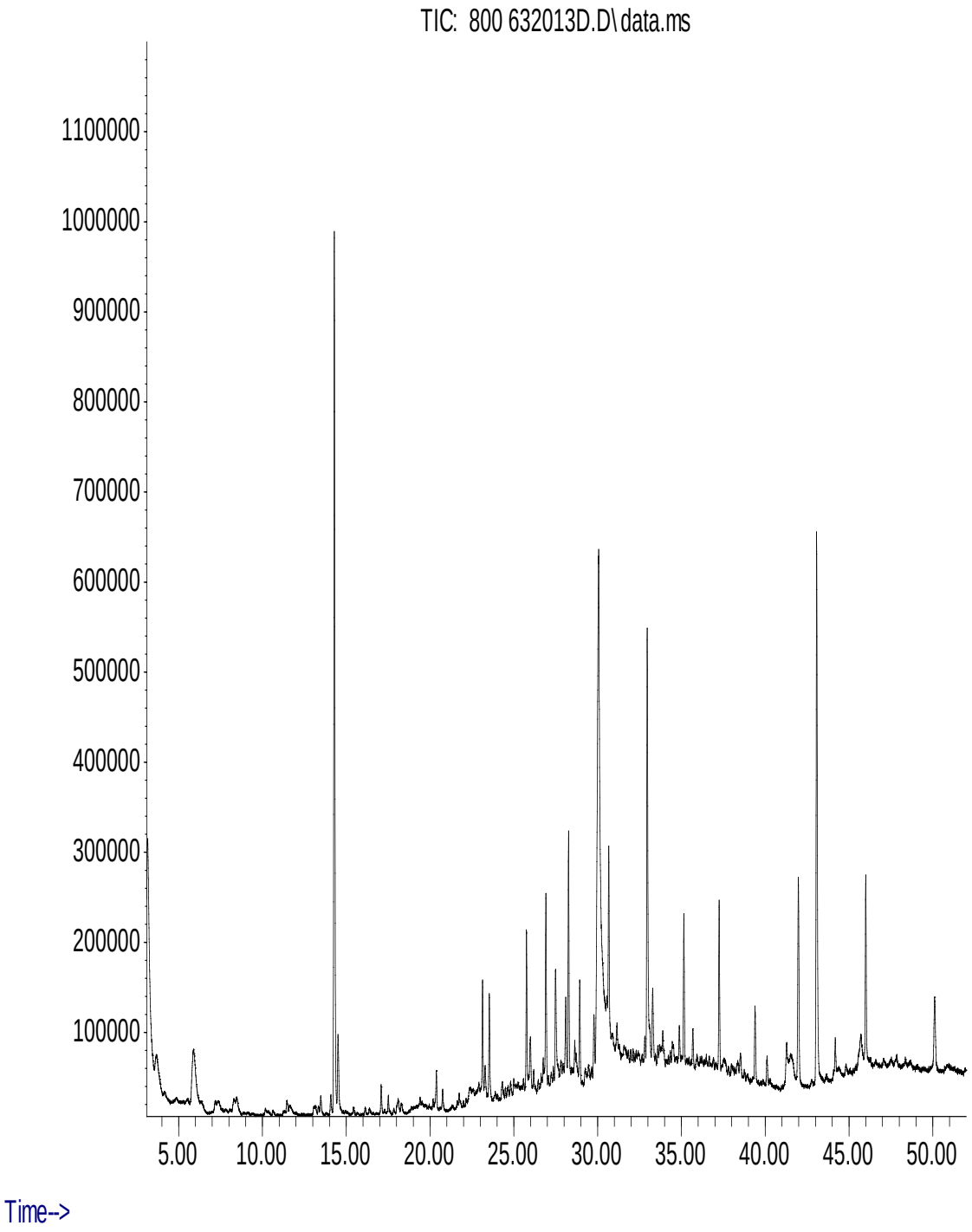


Figure 48: TIC of *Deinbollia pinnata* leaves essential oil collected after 4 hrs

Table 14: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Deinbollia pinnata*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs Cont.
1.	3.69	Benzene,1,2,3-trimethyl -	C_9H_{12}	Methylated benzene		6.21			
2.	11.49	Tridecane	$C_{13}H_{26}$	Long chain alkane		2.19		0.46	
3.	11.62	Formamide, N,N-dibutyl-	$C_9H_{19}NO$ / Dibutyl formamide (DBF)	Amide				1.12	
4.	14.29	4-Tetradecene, (E)	$C_{14}H_{28}$	Long chain alkene					14.93
5.	14.30	1-Dodecene	$C_{12}H_{24}$	Alkene			1.94		
6.	14.31	9-Octadecene, (E)-	$C_{18}H_{36}$	Alkene	4.22			0.52	
7.	14.57	Tetradecane	$C_{14}H_{30}$	Long chain alkane		3.61	1.34	1.64	1.50
8.	17.51	Pentadecane	$C_{15}H_{32}$	Long chain alkane	0.49	2.78	1.36	1.20	
9.	20.38	Hexadecane	$C_{16}H_{34}$	Long chain alkane	2.89	6.39	2.85	2.10	0.74
10.	21.72	Sulfurous acid, butyl dodecyl ester	$C_{16}H_{34}O_3S$	Ester		2.16			
11.	21.75	Heptadecane, 2-methyl -	$C_{18}H_{38}$	Methylated alkane	1.18				
12.	22.34	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	Ketone	0.63	2.12			
13.	23.17	Heptadecane	$C_{17}H_{36}$	Long chain alkane	5.19	7.96	3.54	2.54	
14.	23.29	Dodecane, 2,6,10-trimethyl -	$C_{15}H_{32}$ Farnesan	Methylated alkane		2.32			

Table 14 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Deinbollia pinnata*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs Cont.
15.	23.31	Pentadecane,2,6,10,14-tetra methyl	$C_{19}H_{40}$	Methylated alkane	1.95			1.09	
16.	23.54	Tetradecanal	$C_{14}H_{28}O$ Myristil aldehyde	Aldehyde					1.64
17.	23.97	Benzene,(1-pentyl heptyl)-	$C_{18}H_{30}$	Benzene derivative			1.33	1.34	
18.	24.40	Benzene, (1-propyl nonyl) -	$C_{18}H_{30}$ Dodecane, 4- phenyl	Benzene derivative			1.00	0.95	
19.	25.76	Octadecane	$C_{18}H_{38}$	Long chain alkane	5.78	1.12	2.87	2.07	2.00
20.	26.01	Hexadecane, 2,6, 10,14-tetramethyl-	$C_{20}H_{42}$ Phytane	Methylated alkane	2.74	5.08	1.27		
21.	26.78	Bicyclo[3.1.1] heptane,2,6,6-trimethyl-	$C_{10}H_{18}$ Cis pinane	Methylated alkane	1.28		1.68	1.15	
22.	26.92	2-Pentadecanone, 6,10,14 -trimethyl	$C_{18}H_{36}O$ Perhydrofarnesyl acetone	Methylated secondary ketone	14.19		21.78	15.33	3.16
23.	27.23	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	Ester		1.60			
24.	27.49	Phthalic acid isobutyl nonyl ester	$C_{21}H_{32}O_4$	Ester					2.47

Table 14 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Deinbollia pinnata*

S/N	R/T (min)	Compounds	Formulae / Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs Cont.
25.	27.57	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	Ester			2.79		
26.	28.27	Nonadecane	$C_{19}H_{40}$	Long chain alkane	5.95	3.38	2.53	2.09	4.08
27.	28.93	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ Palmitic acid methyl ester	Carboxylic acid ester				0.41	1.57
28.	29.48	Isophytol	$C_{20}H_{40}O$	Terpenoids	1.41		3.80	4.27	
29.	29.81	Dibutyl phthalate	$C_{16}H_{22}O_4$	Ester	3.42		2.44	1.64	
30	30.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ Palmitic acid	Saturated fatty acid					21.32
31.	30.54	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Ester		15.97			
32.	30.67	Eicosane	$C_{20}H_{42}$	Long chain alkane	6.21	1.59	2.34	2.37	2.43
33.	31.58	1-Docosene	$C_{22}H_{44}$	Alkene		2.06			
34.	32.02	13-Hexyloxacyclo tridec-10-en-2-one	$C_{18}H_{32}O_2$	Unsaturated secondary ketone	2.08				
35.	32.96	Haneicosane	$C_{21}H_{44}$	Long chain alkane	6.65	1.43	2.51	2.16	6.61
36.	33.29	Phytol and isomers	$C_{20}H_{40}O$	Diterpene alcohol			9.90	14.72	1.21

Table 14 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the leaves of *Deinbollia pinnata*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs Cont.
37.	33.82	Heptadecane,2,6,10,15-tetramethyl	$C_{21}H_{44}$	Methylated alkane		1.87			
38.	34.55	Docosane	$C_{22}H_{46}$	Long chain alkane	5.15	1.21	1.83	1.58	
39.	35.14	Heptadecane	$C_{17}H_{36}$	Long chain alkane		1.38		1.58	
40.	35.15	Heneicosane	$C_{21}H_{44}$	Long chain alkane	2.20				2.13
41.	37.26	Tetratriacontane	$C_{34}H_{70}$	Long chain alkane					2.49
42.	39.39	Tetracosane	$C_{24}H_{50}$	Long chain alkane	3.22		1.37	1.09	1.33
43.	41.29	17-Pentatriacontene	$C_{35}H_{70}$	Lone chain alkene					0.46
44.	41.97	Tetratetracontane	$C_{44}H_{90}$	Long chain alkane	1.25				3.75
45.	43.07	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	Ester	3.58	1.31	3.19		10.57
46.	46.01	Tritetracontane	$C_{43}H_{88}$	Long chain alkane				0.89	2.72
47.	50.11	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	Chlorinated alkane	0.90				1.77
TOTAL					**82.56**	**73.74**	**73.66**	**64.31**	**88.88**

The major compounds identified from the leaves of *D. pinnata* were long chain alkanes which include tridecane (2.19, 0.46% in 2^{nd} , 4^{th} hr respectively, R_T 11.49 mins), tetradecane (3.61, 1.34, 1.64, 1.50% in 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 14.57 mins), pentadecane (0.49, 2.78, 1.36, 1.20% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} hr, R_T 17.51 mins), hexadecane (2.89, 6.39, 2.85, 2.01, 0.74% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 20.38 mins), heptadecane (5.19, 7.96, 3.54, 2.54% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} hr, R_T 23.17 mins), octadecane (5.78, 1.12, 2.90, 2.07, 2.00% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} , 4 hrs respectively, R_T 25.76 mins), nonadecane (5.95, 3.38, 2.53, 2.09, 4.08% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 28.22 mins), haneicosane (6.65, 1.43, 2.54, 2.07, 6.61% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 32.96 mins) and docosane (5.15, 1.21, 1.83% in 1^{st}, 2^{nd}, 3^{rd} hr respectively, R_T 34.55 mins); methylated alkanes which include heptadecane, 2-methyl (1.18% in 1^{st} hr, R_T 21.75 mins), dodecane, 2,6,10 – tri methyl (2.32,% in 2^{nd} hr, R_T 23.29 mins), pentane, 2,6,10,14 – tetra methyl (1.95, 1.09% in 1^{st}, 4^{th} hr respectively, R_T 23.31 mins), hexadecane, 2, 6, 10, 14 – tetra methyl (2.74, 5.08, 1.27% in 1^{st}, 2^{nd}, 3^{rd} hr respectively, R_T 26.01 mins), bicyclo[3.1.1] heptane,2,6,6- tri methyl- (1.28, 1.68, 1.15% in 1^{st}, 3^{rd}, 4^{th} hr respectively, R_T 26.78 mins), heptane, 2, 6, 10,15 – tetra methyl (1.87% in 2^{nd} hr, R_T 33.82 mins). Esters were also identified and they include sulfurous acid, butyl dodecyl ester (2.16% in 2^{nd} hr, R_T 21.72 mins), octatriacontyl pentafluoropropionate (1.60% in 2^{nd} hr, R_T 27.23 mins), phthalic acid isobutyl nonyl ester (2.47% in 4 hr, R_T 27.47 mins), phthalic acid, butyl tetradecyl ester (2.79% in 3^{rd} hr, R_T 27.57 mins), hexadecanoic acid, methyl ester (0.41, 1.57% in 4^{th}, 4 hrs respectively, R_T 28.93 mins), dibutyl phthalate (3.42, 2.44% in 1^{st}, 3^{rd} hr respectively, R_T 29.81 mins) and hexadecanoic acid, ethyl ester (15.97% in 2^{nd} hr, R_T 30.54 mins). Others were long chain alkenes which include 4 – tetradecane (E) (14.93% in 4 hr, R_T 14.29 mins), 1 – dodecene (1.94% in 3^{rd} hr, R_T 14.30 mins), 9 – octadecene, (E) – (4.22, 0.52% in 1^{st}, 4^{th} hr, R_T 14.31 mins), 1 – docosene (2.06% in 2^{nd} hr, R_T 31.58 mins); diterpene alcohol which include isophytol (1.41, 3.84, 4.27% in 1^{st}, 3^{rd}, 4^{th} hr respectively, R_T

29.48 mins) and phytol (10.00, 11.10, 1.21% in 3rd, 4th, 4 hrs respectively, R_T 33.29 mins); benzene derivatives which include benzene,1,2,3-trimethyl – (6.21% in 2nd hr, R_T 3.69 mins), benzene,(1-pentyl heptyl)- (1.33, 1.34% in 3rd, 4th hr respectively, R_T 23.97 mins) and benzene, (1-propyl nonyl)- (1.00, 0.95% in 3rd, 4th hr respectively, R_T 24.40 mins); ketones which include 2-piperidinone, N-[4-bromo-n-butyl]- (0.63, 2.12% in 1st, 2nd hr respectively, R_T 22.34 mins) and 13-hexyloxacyclotridec-10-en-2-one (2.08% in 1st hr, R_T 32.02 mins). Tetradecanal, an aldehyde (1.64% in 4 hrs, R_T 23.54 mins) was also identified. A methylated secondary ketone, 2-pentadecanone, 6, 10, 14 – trimethyl (14.19, 21.78, 14.68, 3.16% in 1st, 3rd, 4th, 4 hrs respectively, R_T 26.92 mins) was identified in high yield.

4.2.6 GC-MS Analyses of the Essential oil Samples from the aerial parts of *Euphorbia hirta*

The chromatograms obtained from the GC-MS analyses of the essential oil samples from the 1st hourly fraction and 4 hrs single collection of *Euphorbia hirta* are shown in Figures 49 and 50 and the retention times, names, formulae, classes and percentage abundances of the various constituent compounds are presented in Table 15.

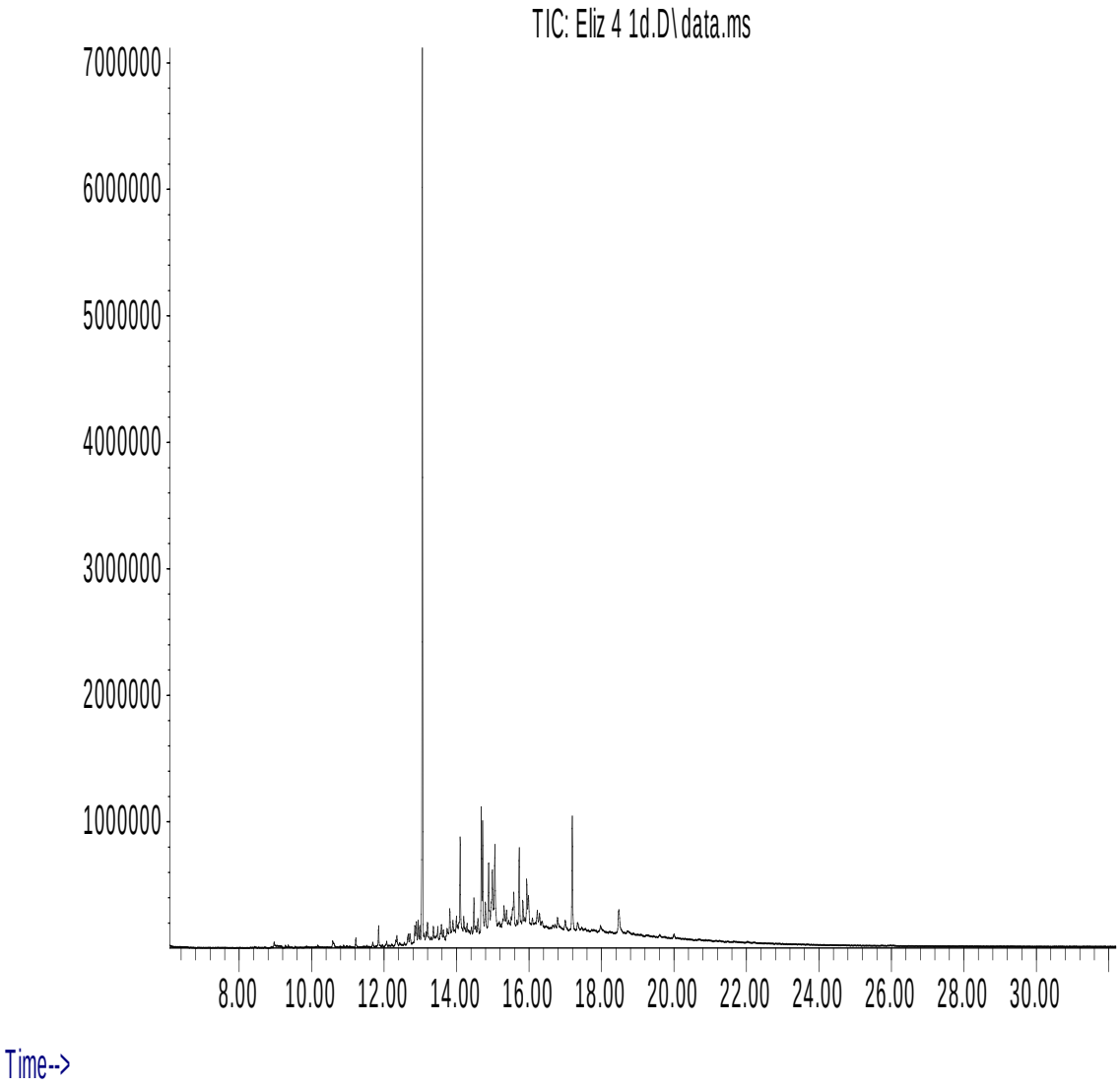


Figure 49: TIC of *Euphorbia hirta* aerial parts essential oil collected after 1st hr

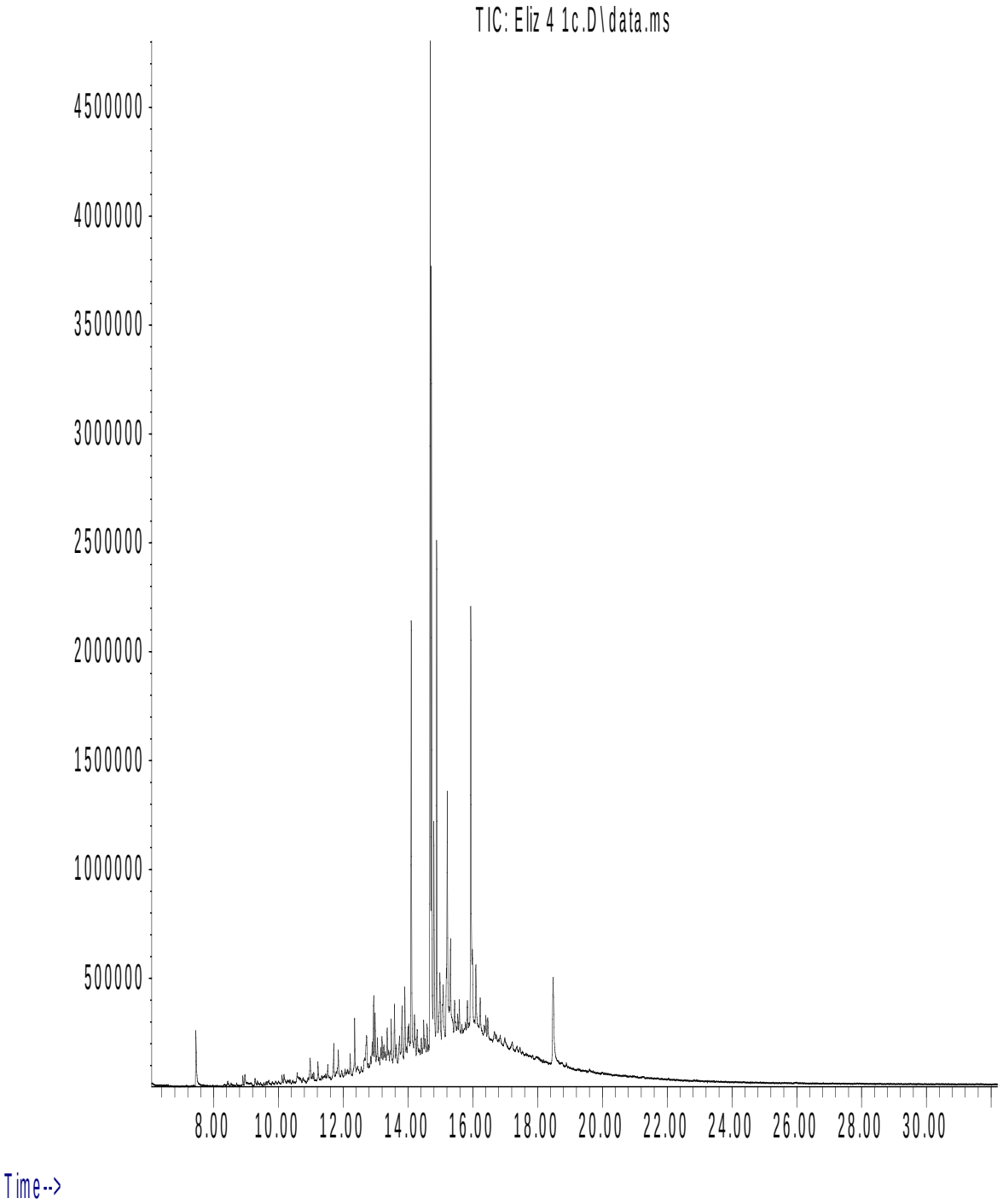


Figure 50: TIC of *Euphorbia hirta* aerial parts essential oil collected after 4 hrs

Table 15: Compounds obtained from the GC-MS analyses of essential oil samples from the aerial parts of *Euphorbia hirta*

S/N	R/T (min)	Compounds	Formulae / Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
1.	7.46	2-Butoxy ethanol	$C_6H_{14}O_2$	Alcohol					1.13
2.	11.22	2-Propenoic acid, 2-ethylhexyl ester	$C_{11}H_{20}O_2$	Ester			5.96	1.52	
3.	12.36	Tetradecane	$C_{14}H_{30}$	Long chain alkane					1.01
4.	12.94	Tetradecane, 2,6,10-trimethyl	$C_{17}H_{36}$	Methylated alkane	0.90				1.24
5.	13.06	N.I.			32.31	14.00			
6.	13.59	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	Fatty acid alcohol					1.18
7.	13.82	Ledene oxide – (11)	$C_{15}H_{24}O$	Sesquiterpene	1.52				
8.	13.91	2-Methyl - 1- hexadecanol	$C_{17}H_{36}O$	Methylated fatty acid alcohol					1.40
9.	14.10	Hexadecanal	$C_{16}H_{32}O$ Palmitaldehyde	Fatty acid aldehyde	5.23				7.63
10.	14.10	Octadecanal	$C_{18}H_{36}O$ Stearylaldehyde	Fatty acid aldehyde		5.99	5.01	4.45	
11.	14.48	Phenol 2,6-bis(1,1-dimethylethyl) -4-ethyl	$C_{16}H_{26}O$ Ionol 2	Phenol derivative	1.46	4.90	1.00	0.82	0.62
12.	14.69	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ Phytol	Diterpene alcohol	4.12	10.55	7.39	7.86	13.31

N.I. = Not Identified

Table 15 contd: Compounds obtained from the GC-MS analyses of essential oil samples from the aerial parts of *Euphorbia hirta*

S/N	R/T (min)	Compounds	Formular/ Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
13.	14.73	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	Methylated secondary ketone	4.65	8.45	8.13	7.32	12.37
14.	14.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ /Phytol	Diterpene alcohol	1.89	7.80	4.31	2.96	4.74
15.	14.88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ /Phytol	Diterpene alcohol	3.82	7.81	5.45	4.80	8.41
16.	15.06	N.I			5.11	7.60	3.24	1.50	2.53
17.	15.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ Palmitic acid	Saturated fatty acid				18.48	6.26
18.	15.31	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	Ester	2.11		3.52	3.95	3.88
19.	15.44	Oleic acid	$C_{18}H_{34}O_2$	Unsaturated fatty acid					1.42
20.	15.94	Phytol	$C_{20}H_{40}O$	Diterpene alcohol	2.21	3.85	3.82	6.57	8.29
21.	18.48	1,2-Benzenedicarboxylic acid diiso octyl ester	$C_{24}H_{38}O_4$	Ester	2.29	7.28			2.90
TOTAL					**67.62**	**78.23**	**47.83**	**60.23**	**78.32**

N.I. = Not Identified

The major compounds identified in the essential oil fractions of *E. hirta* aerial parts were 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer (5.71, 26.16, 9.76, 10.82, 26.46% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 14.69, 14.80 and 14.88 mins) which are methylated unsaturated long chain fatty acid alcohols, 2-pentadecanone-6,10,14-trimethyl-, a methylated long chain secondary ketone (4.65, 8.45, 8.13, 7.32, 12.37% in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 14.73 mins), phytol which is an isomer of 3,7,11,15-tetramethyl-2-hexadecene-1-ol (2.21, 3.85, 3.82, 6.57, 8.29%, in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 4 hrs respectively, R_T 15.94 mins), n-hexadecanoic acid, a saturated fatty acid, (18.48, 6.26% in 4^{th}, 4 hrs respectively, R_T 15.22 mins) and hexadecanal (7.63% in 4 hrs, R_T 14.10 mins) and octadecanal (5.99, 5.01, 4.45% in 2^{nd}, 3^{rd}, 4^{th} hr respectively, R_T 14.10 mins) which are long chain fatty acid aldehydes. The remaining compounds were esters namely phthalic acid butyltetradecyl ester (3.52, 3.95, 3.88% in 3^{rd}, 4^{th}, 4 hrs respectively, R_T 15.313 mins), 1,2-benzene dicarboxylic acid diisooctyl ester (2.29, 7.28, 2.90% in 1^{st}, 2^{nd}, 4 hrs respectively, R_T 18.48 mins) and 2-propenoic acid, 2-ethylhexyl ester (5.96, 1.52% in 3^{rd}, 4^{th} hr respectively, R_T 11.22 mins); oleic acid, an unsaturated fatty acid (1.42% in 4 hr, R_T 15.44 mins), 2-methyl hexadecanol, a methylated fatty acid alcohol (1.40% in 4 hr, R_T 13.91 mins), tetradecane, 2,6,10-trimethyl, a methylated long chain alkane (0.90, 1.24% in 1^{st}, 4 hr, R_T 12.94 mins), 13-heptadecyn-1-ol, an unsaturated alcohol (1.18% in 4 hr, R_T 13.59 mins), 2-butoxy ethanol, an alcohol (1.13% in 4 hrs, R_T 7.46 mins), tetradecane, a long chain alkane, (1.01% in 4 hr, R_T 12.36 mins), Ledene oxide – (11) (1.52% in 1^{st} hr, R_T 13.82 mins) and phenol 2,6-bis(1,1-dimethylethyl)-4-ethyl (1.46, 4.90% in 1^{st}, 2^{nd} hr respectively, R_T 14.48 mins).

4.2.7 GC-MS Analyses of the Essential oil Samples from the roots of *Pterocarpus osun*

The chromatograms obtained from the GC-MS analyses of the essential oil samples from the 1st, 2nd, 3rd hourly fractions and 4 hr single collection of *Pterocarpus osun* are shown in Figures 51 - 54 and the retention times, names, formulae, classes and percentage abundances of the various constituent compounds are presented in Table 16.

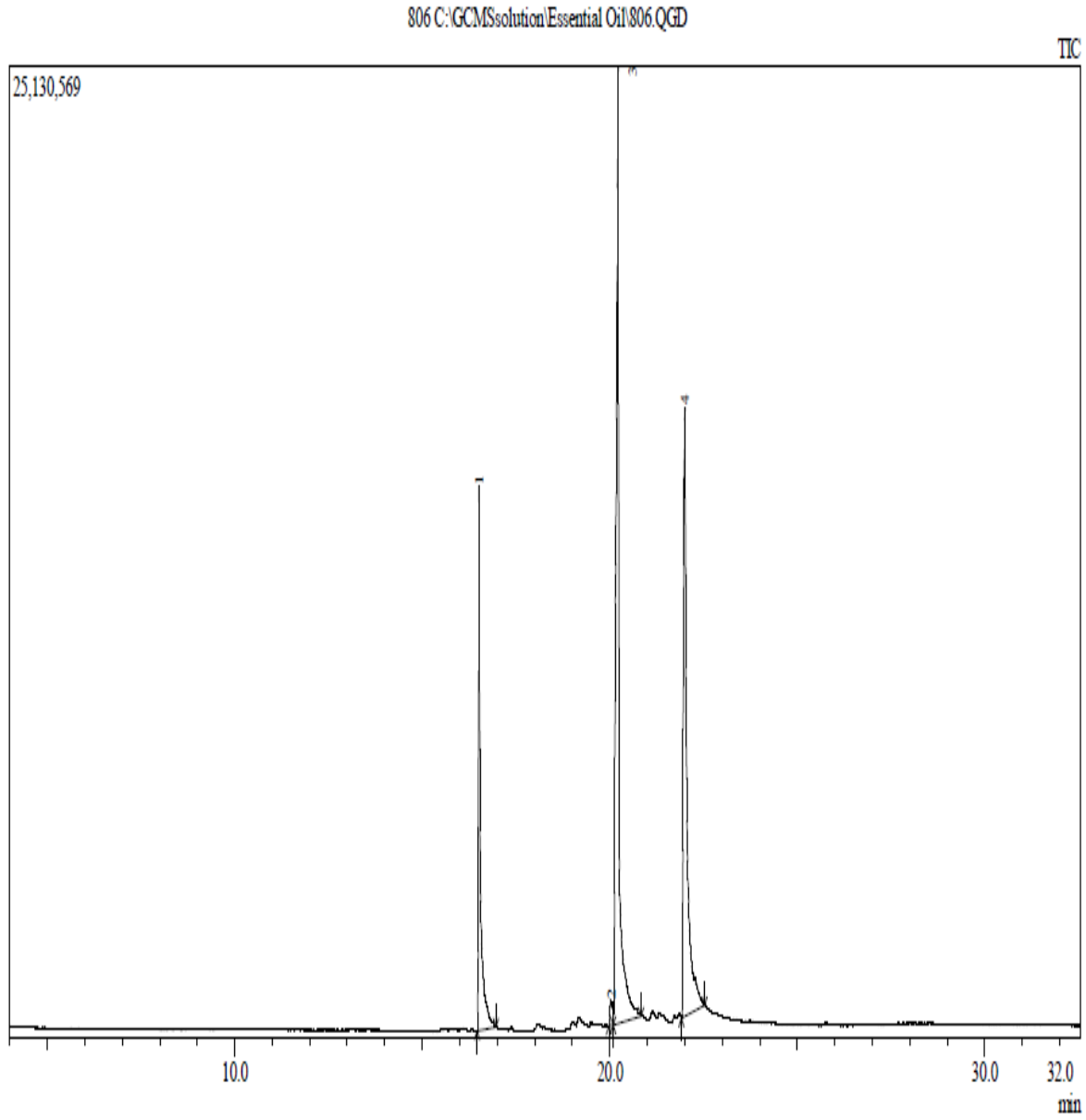


Figure 51: TIC of *Pterocarpus osun* root essential oil collected after 1st hr

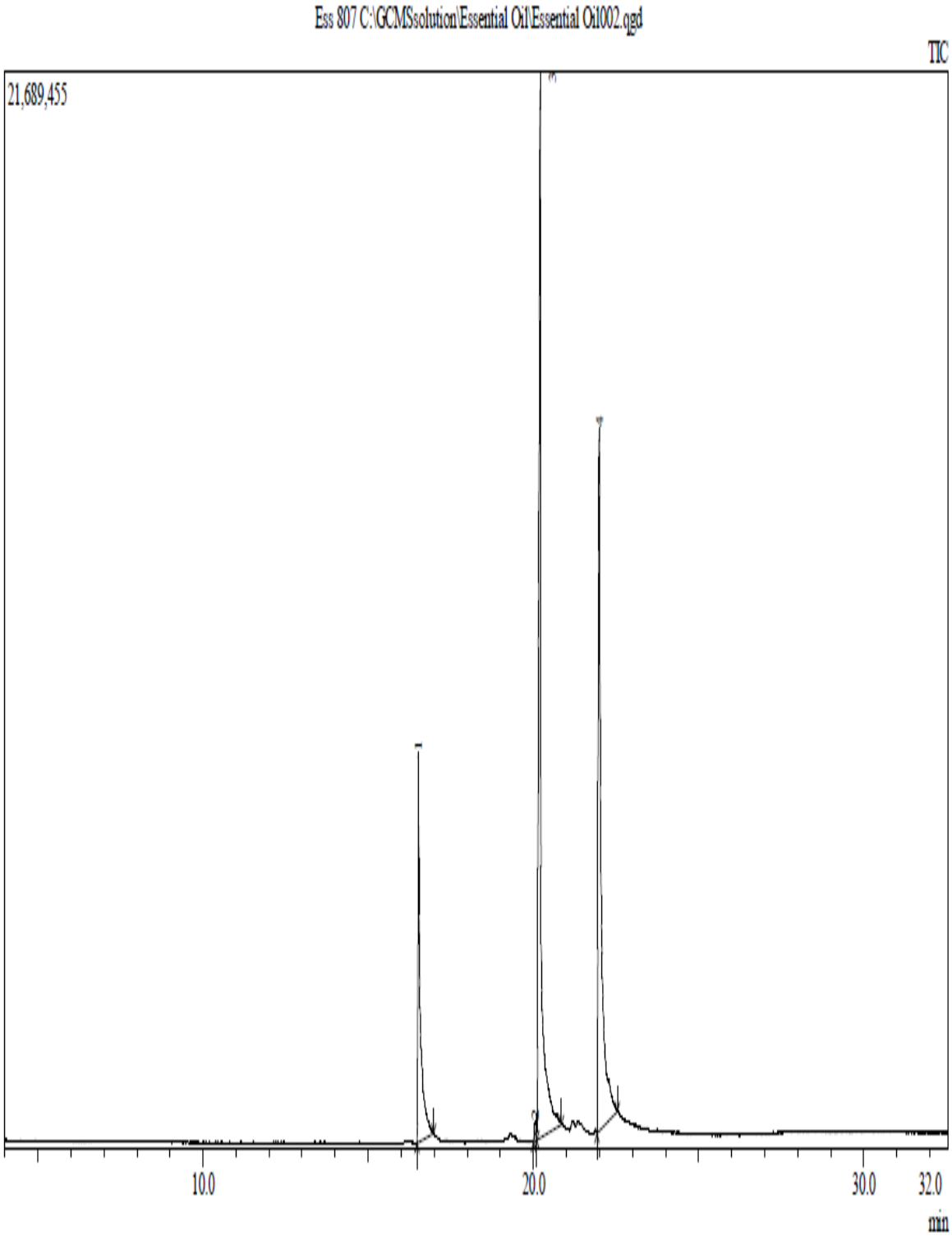


Figure 52: TIC of *Pterocarpus osun* root essential oil collected after 2nd hr

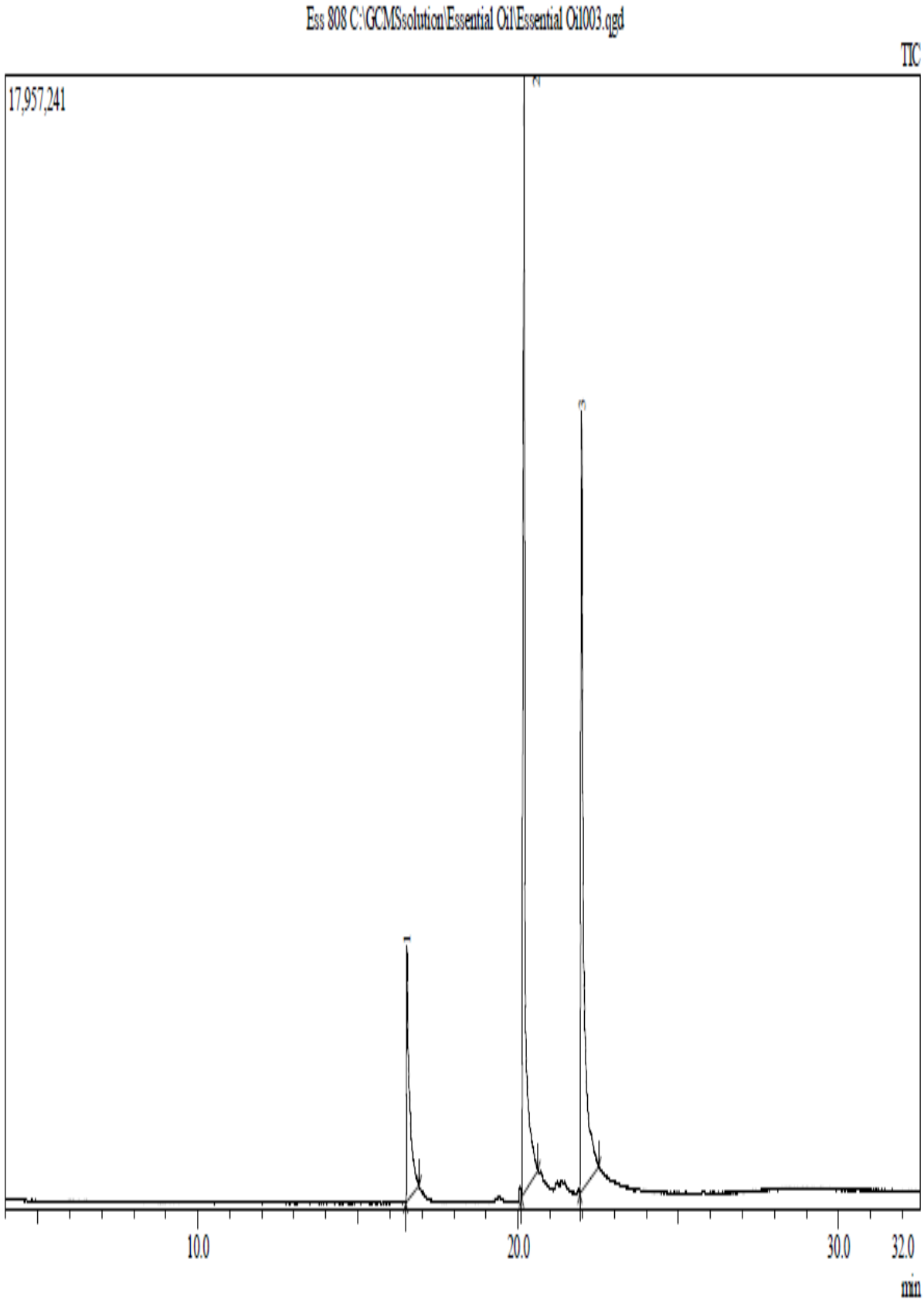


Figure 53: TIC of *Pterocarpus osun* root essential oil collected after 3rd hr

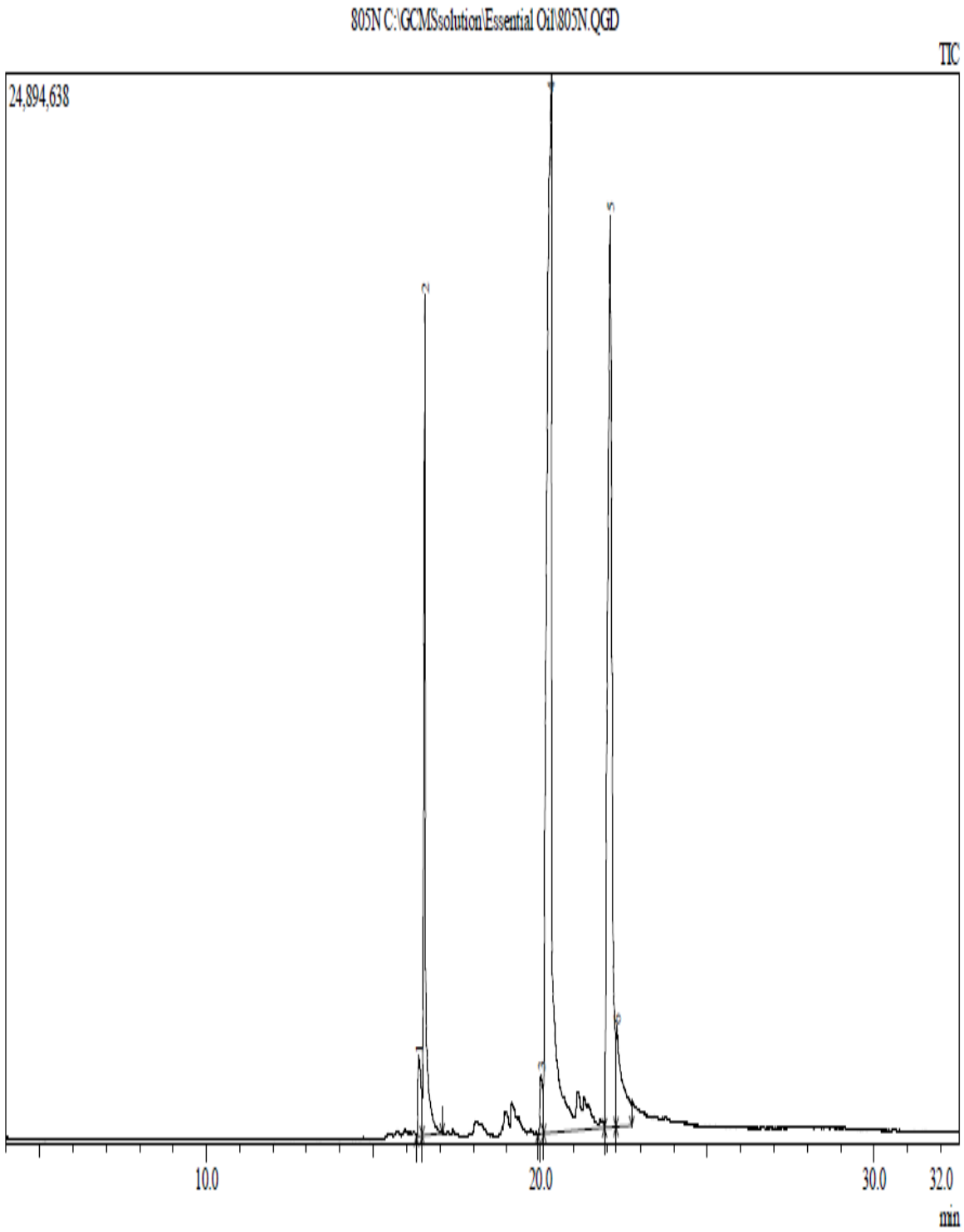


Figure 54: TIC of *Pterocarpus osun* root essential oil collected after 4 hrs

Table 16: Compounds obtained from the GC-MS analyses of essential oil samples from the roots of *Pterocarpus osun*

S/N	R/T (min)	Compounds	Formulae/Other Names	Classes	Percentage of Total				
					1st hr	2nd hr	3rd hr	4th hr	4 hrs cont.
1.	16.51	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$/ Elemicin	Benzene derivative	16.75	16.25	14.68	12.80	12.48
2.	20.03	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	Long chain fatty acid					1.33
3.	20.03	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	Methylated unsaturated fatty acid	0.96				
4.	20.05	9-Octadecene, 1,1-dimeth oxy-, (Z)-	$C_{20}H_{40}O_2$/ Olealdehyde	Long chain methoxy alkane		0.59			
5.	20.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$/ Palmitic acid	Saturated fatty acid	48.79	46.84	44.24	43.93	48.58
6.	22.00	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$/Linolei c acid	Unsaturated fatty acid	33.50	36.31	41.08	43.27	30.84
7.	22.30	17-Octadecynoic acid	$C_{18}H_{32}O_2$	Unsaturated fatty acid					4.85
TOTAL					**100.00**	**99.99**	**100.00**	**100.00**	**98.08**

Generally, seven compounds were identified from the five different fractions of essential oils obtained from the root of *Pterocarpus osun*. Three of these compounds appeared in all the fractions of the essential oil with high percentage of the total constituents and they were n-hexadecanoic acid, a saturated fatty acid (48.79, 46.84, 44.24, 43.93, 48.58% in 1st, 2nd, 3rd, 4th, 4 hrs respectively, R_T 20.23 mins), 9,12-octadecadienoic acid (Z,Z)-, an unsaturated fatty acid (33.50, 36.31, 41.08, 43.27, 30.84% in 1st, 2nd, 3rd, 4th, 4 hrs respectively, R_T 22.00 mins) and benzene, 1,2,3-trimethoxy-5-(2-propenyl)- (16.75, 16.25, 14.68, 12.80, 12.48% in 1st, 2nd, 3rd, 4th, 4 hrs respectively, R_T 16.51 mins). These major compounds add up to 99.04, 99.40, 100.00, 100.00 and 91.86% of total constituents in 1st, 2nd, 3rd, 4th, 4 hrs fractions respectively. The remaining four minor constituents were 9-hexadecenoic acid (1.33% in 4 hr, R_T 20.03 mins), Z-8-methyl-9-tetradecenoic acid (0.96% in 1st hr, R_T 20.03 mins), 9-octadecene, 1,1-dimethoxy-, (Z)- (0.59% in 2nd hr, R_T 20.05 mins) and 17-Octadecynoic acid (4.85% in 4 hr, R_T 22.30 mins). The 3rd and 4th fractions of the essential oils had none of the minor constituents hence the major compounds added up to 100% of the total constituents in each case.

4.2.8 Known Bioactive Compounds in the Essential Oil Fractions of the Plants

Many compounds identified from the essential oil samples of the plants under study have known bioactivities which include anti-inflammatory, antioxidant and antibacterial activities relevant for the management of asthma. The medicinal plants with their bioactive compounds relevant to asthma and their percentage of total constituents are recorded on Table 17.

Table 17: Percentage composition of some bioactive compounds in the essential oils of the medicinal plants and their relevance to asthma

S/N	Compounds	Medicinal plants							Relevance to Asthma
		AD	**CP**	***CL***	**DM**	**DP**	**EH**	***PO***	
1.	Phytol & isomers	8.27	42.71	10.25	37.75	10.73	34.75	-	Anti-inflammatory (Silva *et al.,* 2014; Hulqvist *et al*., 2006). Antitumor (Kim *et al.,* 2015)
2.	n-Hexadecanoic acid	54.71	-	40.35	2.64		6.26	48.58	Anti-inflammatory (Aperna *et al.,* 2012)
3.	Poly Unsaturated Fatty Acid	7.28	-	4.66	3.98	-	-	-	Anti-inflammatory (Zhao *et al.,* 2005) Antioxidant (Yessoufou *et al.,* 2015)
4.	Oleic acid	-	-	-	-	-	1.42	-	Anti-inflammatory [Aparicio, R., & Harwood, J. (2013)], Antioxidant (Cho *et al*., 2010) Antitumor (Carrillo *et al*., 2012)
5.	Squalene	2.69	-	-	-	-	-		Anti-inflammatory (Cárdeno *et al.,* 2015) Antitumor (Desai *et al.,* 1996); Antioxidant (Khono *et al.,*1995)
6.	2-Pentadecanone, 6,10, 14-trimethyl (Perhydrofarnesyl acetone)	4.02	25.01	10.49	10.66	3.16	28.55	-	Anti-inflammatory (Sjögren *et al*., 1999)
	TOTAL	**76.97**	**67.72**	**65.75**	**55.03**	**13.89**	**70.98**	**48.58**	

4.2.9 The mass spectra with the structures of some bioactive compounds in the essential oils of the medicinal plants relevant to asthma

The mass spectra with the structures of some bioactive compounds in the essential oils of the medicinal plants relevant to asthma are shown in Figures 55 – 59.

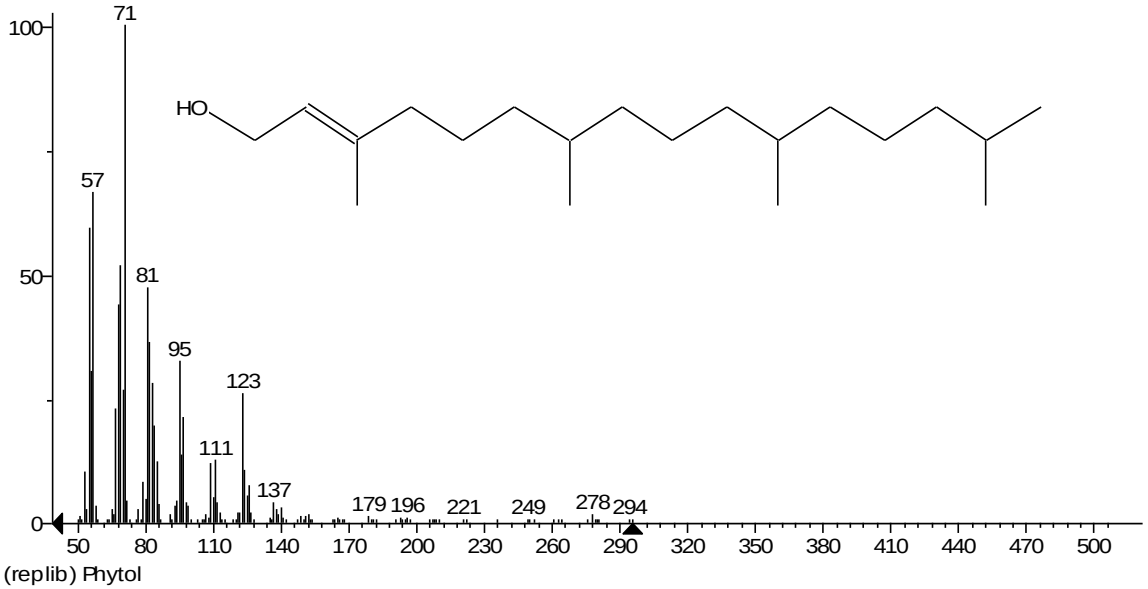


Figure 55: The mass spectrum and structure of phytol

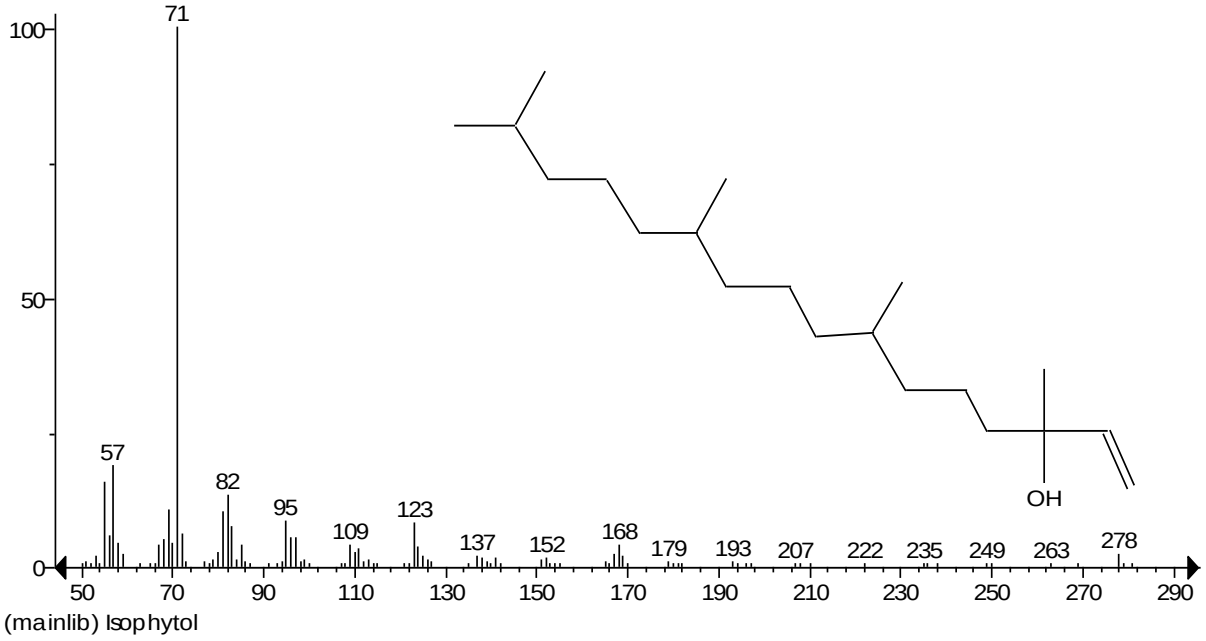


Figure 56: The mass spectrum and structure of isophytol

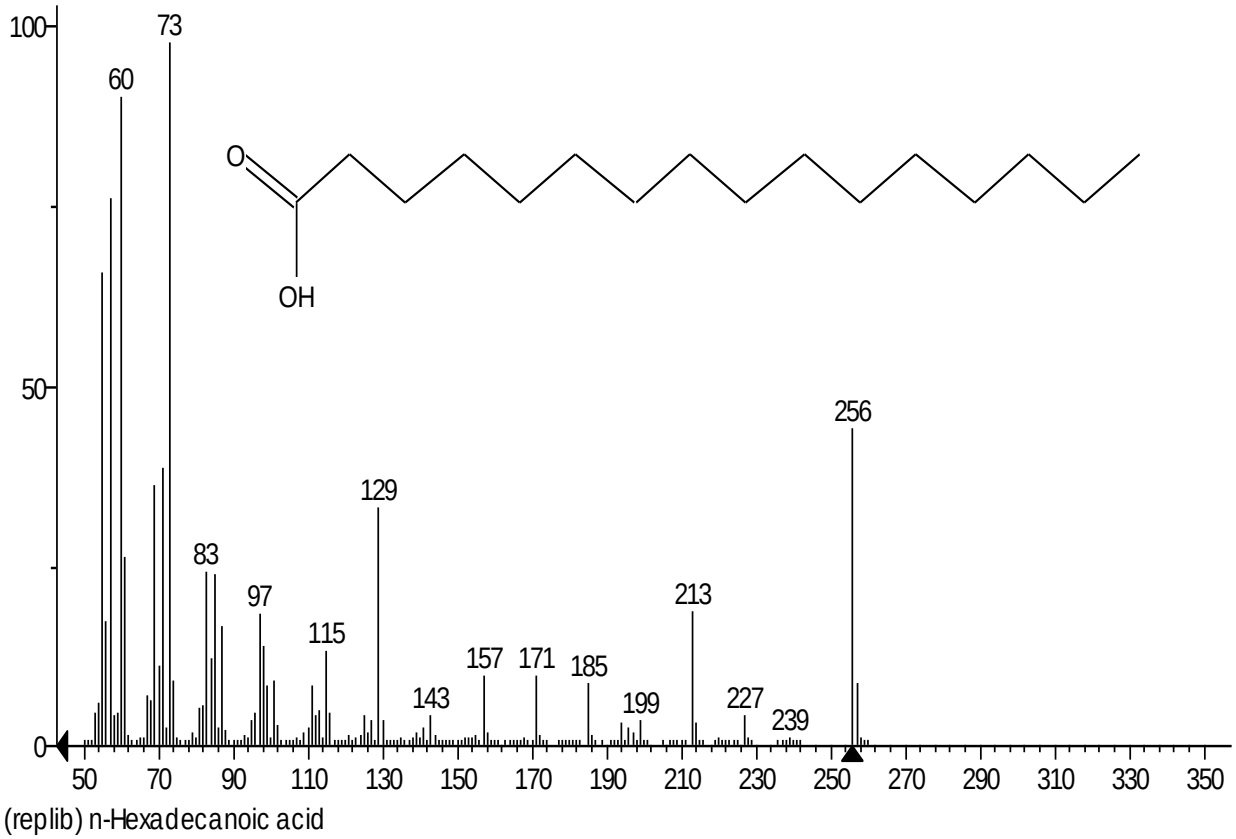


Figure 57: The mass spectrum and structure of n-hexadecanoic acid

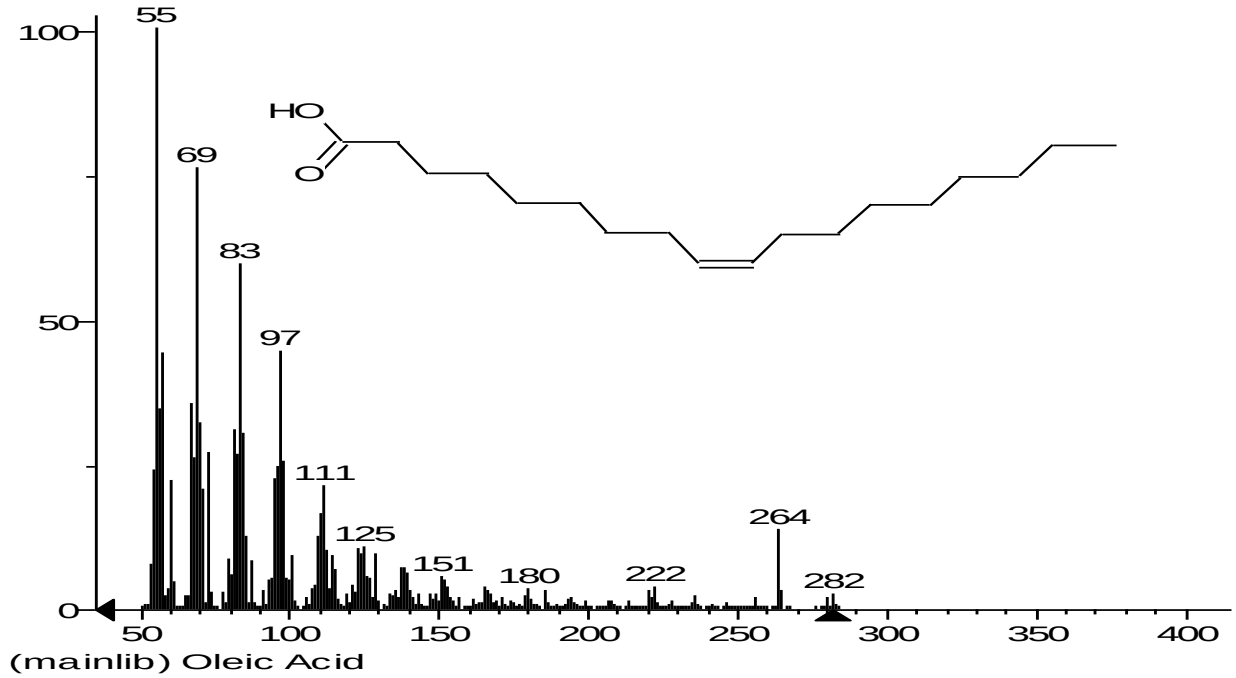


Figure 58: The mass spectrum and structure of oleic acid

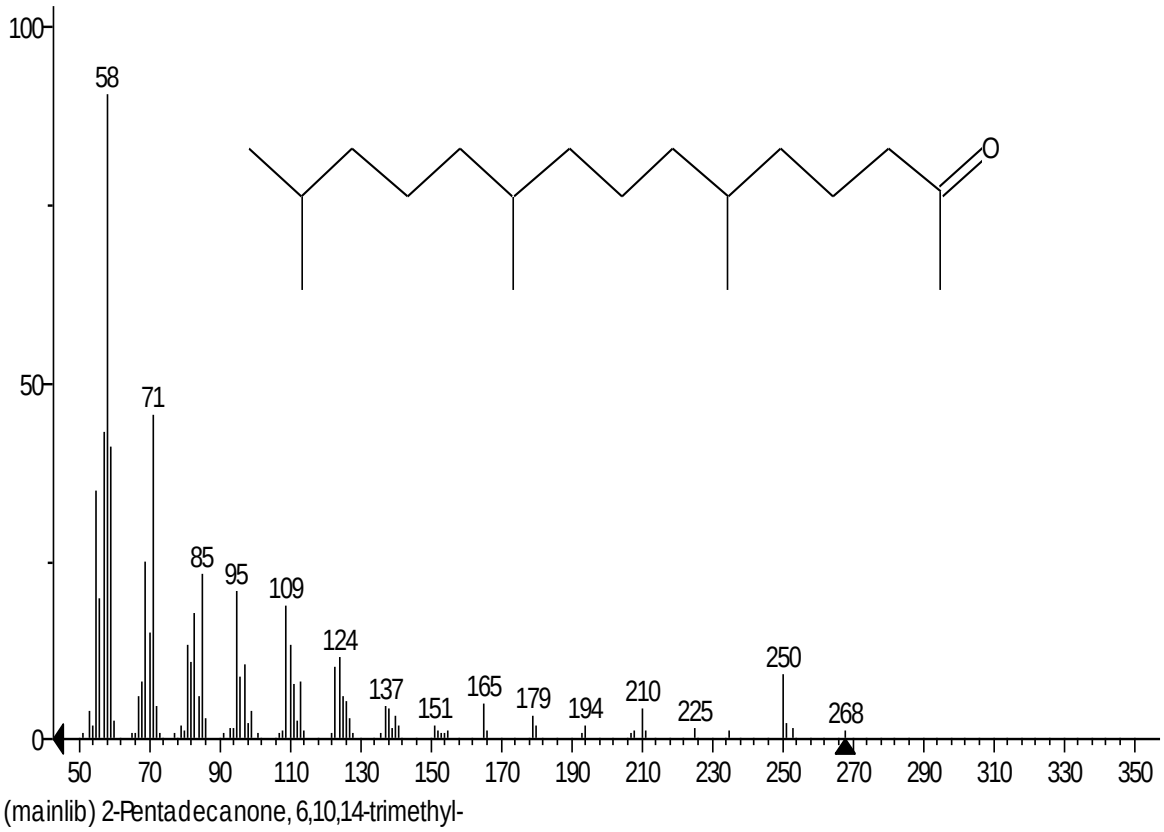


Figure 59: The mass spectrum and structure of 2-Pentadecanone, 6,10,14-trimethyl-

4.2.10 The mass spectra with the structures of some other compounds identified in the medicinal plants

The mass spectra with the structures of some of the other compounds identified in the medicinal plants are presented in Figures 60 -67.

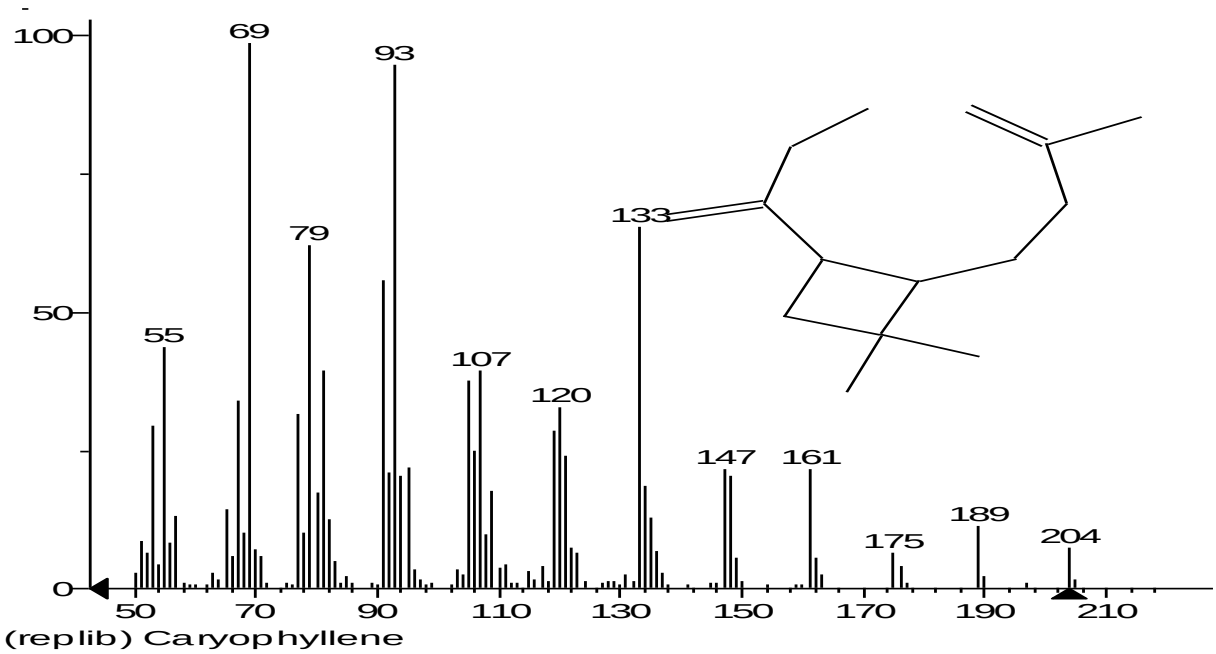


Figure 60: The mass spectrum and structure of caryophyllene

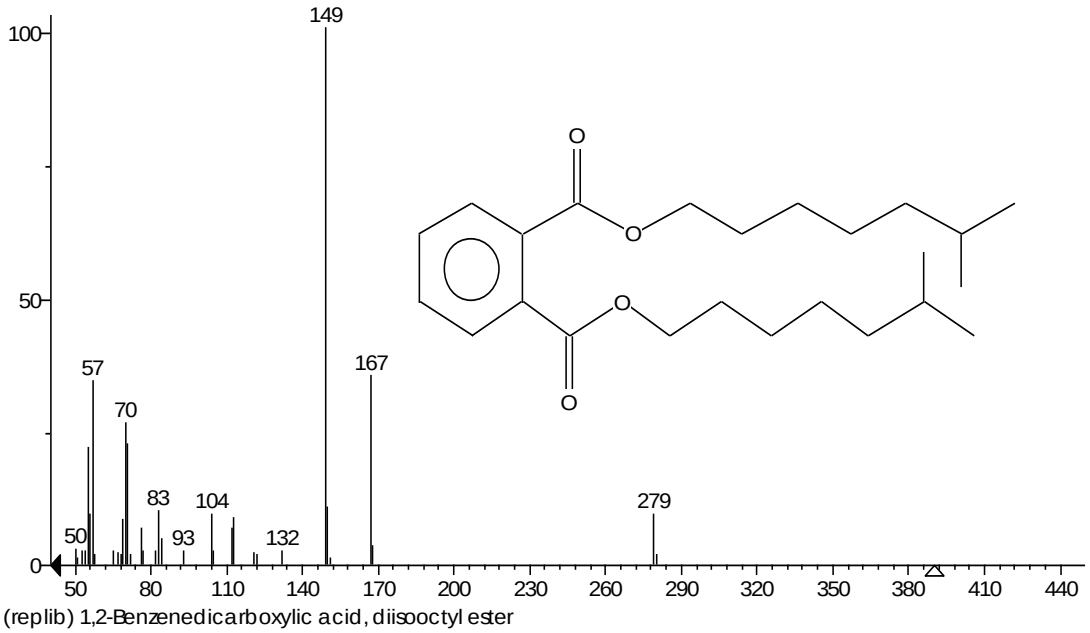


Figure 61: The mass spectrum and structure of 1,2-Benzenedicarboxylic acid, diisooctyl ester

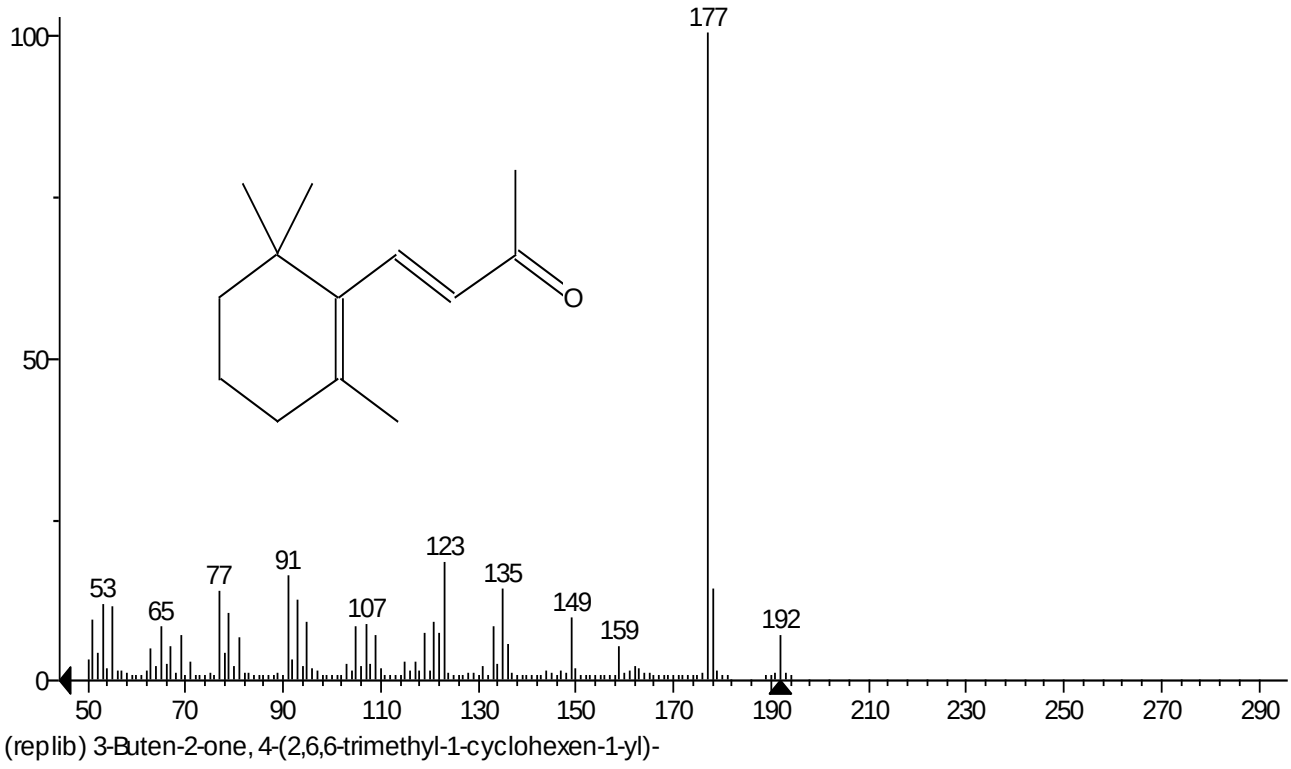


Figure 62: The mass spectrum and structure of 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-

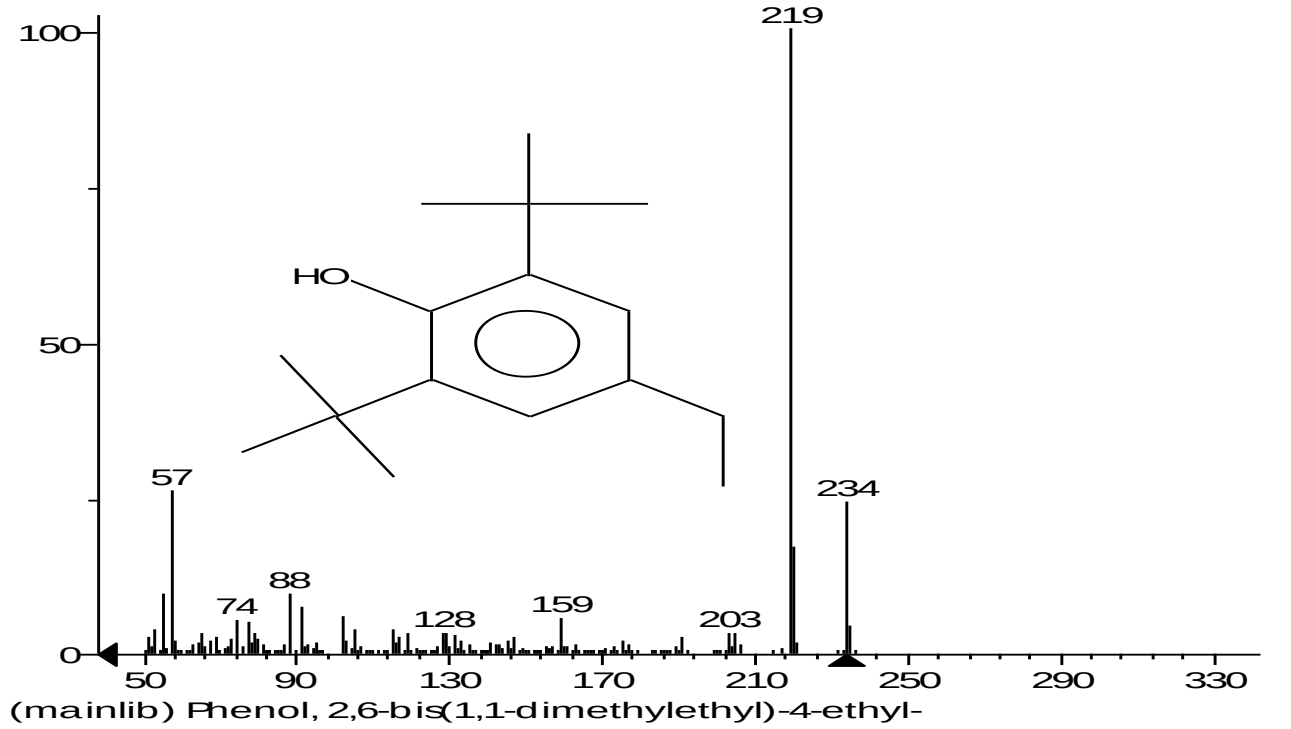


Figure 63: The mass spectrum and structure of phenol, 2,6-bis(1, 1-dimethylethyl)-4-ethyl-

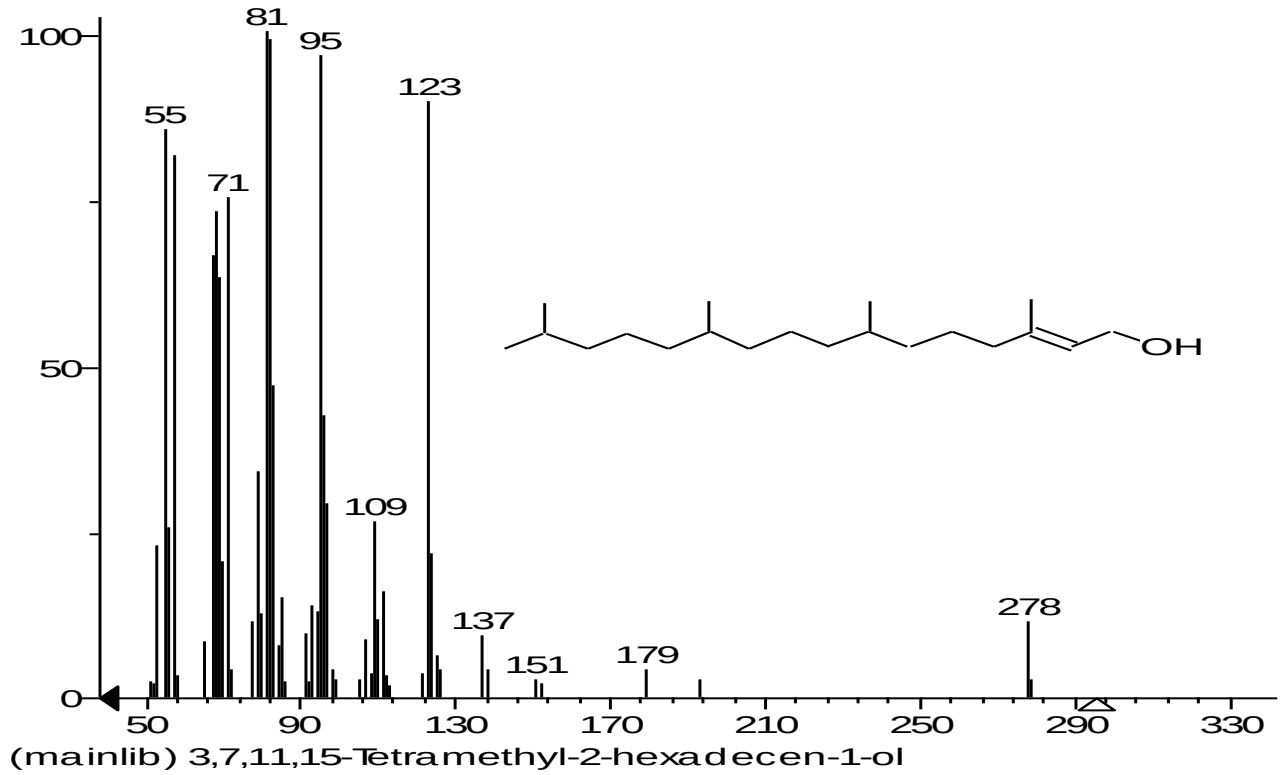


Figure 64: The mass spectrum and structure of 3,7,11,15-tetramethyl-2-hexadecen-1-ol

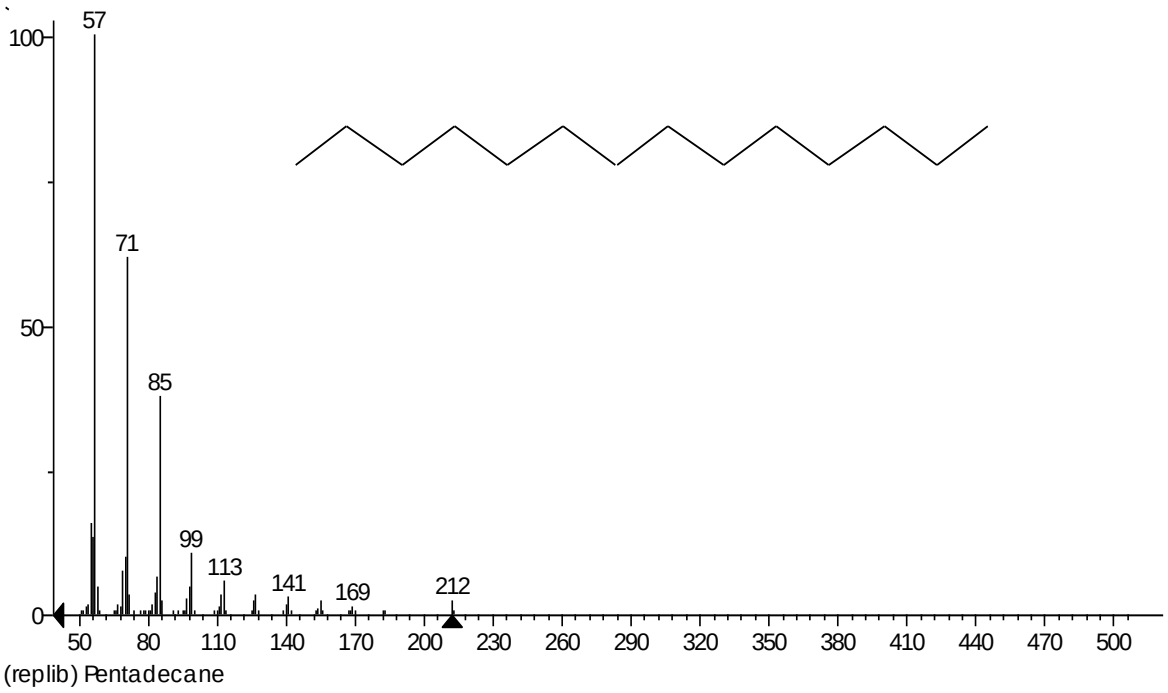


Figure 65: The mass spectrum and structure of pentadecane

Figure 66: The mass spectrum and structure of hexadecanal

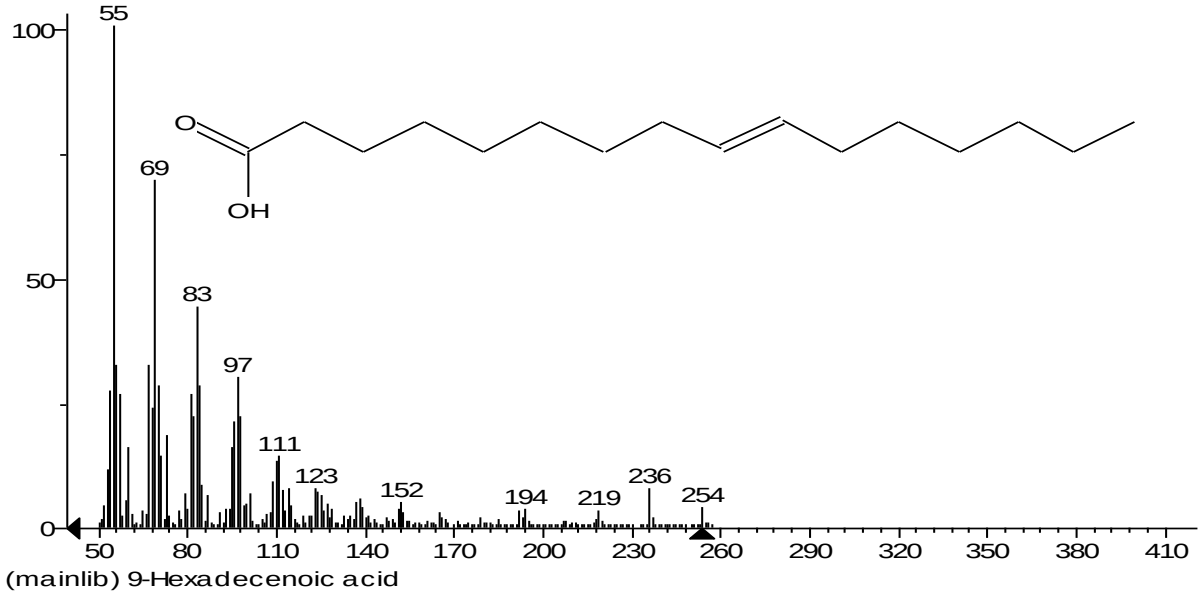


Figure 67: The mass spectrum and structure of 9-hexadecenoic acid

The percentage total of the bioactive compounds as obtained from the library of the mass spectrometer were compared and *Adansonia digitata*, *Calotropis procera* and *Euphorbia hirta* have the highest values hence were chosen for further investigation

.

4.3 The 50% aqueous Methanol Extraction/Fractionation and Soxhlet Exraction

Fifty percent aqueous methanol was used to extract the extractable components of *Adansonia digitata* (AD), *Calotropis procera* (CP) and *Euphorbia hirta* (EH) and fractionation of the extracts was done using non-polar solvent, hexane (hex), medium polar, ethyl acetate (EtOAc) and polar solvent, butanol (BuOH). The results are shown in Table 18. Generally, the extraction recorded a low yield of crude extracts (2.93 - 8.43%), hexane fraction (3.8 - 8.6% x 10^{-3}), EtOAc fraction (0.09 - 0.13%) and BuOH fraction (0.18 - 0.23%). Due to the very low yield of 50% aq.MeOH extraction, another extraction was carried out using Soxhlet apparatus with hexane, dichloromethane (DCM) and MeOH. The average yield was in the range of 0.82-6.17% for the extracts. The yields are recorded in Table 18.

Table 18: Percentage yield of 50% aq. MeOH crude extracts with fractions and Soxhlet extracts

Plants	**% Yield**						
	50% aq. MeOH crude extract	**Fractions from 50% aq. MeOH**			**Soxhlet**		
		$(Hex)_m$	**EtOAc**	**BuOH**	**Hex**	**DCM**	**MeOH**
A. digitata	8.43	0.0038	0.160	0.228	2.74	1.34	6.17
C. procera	4.49	0.0071	0.091	0.194	3.42	1.68	4.22
E. hirta	2.93	0.0086	0.131	0.175	2.92	0.82	3.34

$(Hex)_m$ = Hexane fraction from 50% methanol extract; EtOAc = ethylacetate;
BuOH = butanol; Hex = hexane; DCM = dichloromethane; MeOH = methanol

4.4 The Solid Phase Extraction (SPE) of the Methanol Extracts of the Plants

The percentage yield of fractions obtained from the SPE of AD, CP and EH methanol extracts using 20, 40, 60, 80 and 100% MeOH are recorded on Table 19. The different Solid Phase extracts were obtained in moderate to good yield in the range of 45.59 – 68.43%.

Table 19: Percentage yield of fractions from soxhlet methanol extracts obtained using Solid Phase Extraction (SPE)

S/N	**Concentration of aq. MeOH (%)**	**% Yield**		
		Adansonia digitata	***Euphorbia hirta***	***Calotropis procera***
1.	20	29.40	33.90	27.15
2.	40	28.65	5.25	5.89
3.	60	1.45	3.53	1.62
4.	80	2.23	3.26	1.28
5.	100	6.70	10.89	9.65
Total		68.43	56.83	45.59

4.5 HPLC analyses of the SPE fractions obtained from the leaves of *A. digitata, C. procera* and aerial parts of *E. hirta*

The representative results of HPLC-PDA analyses of the solid phase extraction (SPE) fractions of *A. digitata, C. procera* and aerial parts of *E. hirta* are presented in Figures 68 – 79 and Tables 20 – 22.

4.5.1 HPLC analyses of the SPE fractions obtained from the leaves of *A. digitata*

The HPLC chromatogram for the SPE fractions obtained from the leaves of *A. digitata* is presented in Figure 68. Major peaks were observed at retention times 2.48, 2.58, 9.27 and 15.74 minutes. These with the peak heights, areas and relative areas of the peaks obtained are shown in Table 20 while the representative UV-Vis spectra showing different absorption maxima from the scanned peaks are presented in Figure 69 - 71.

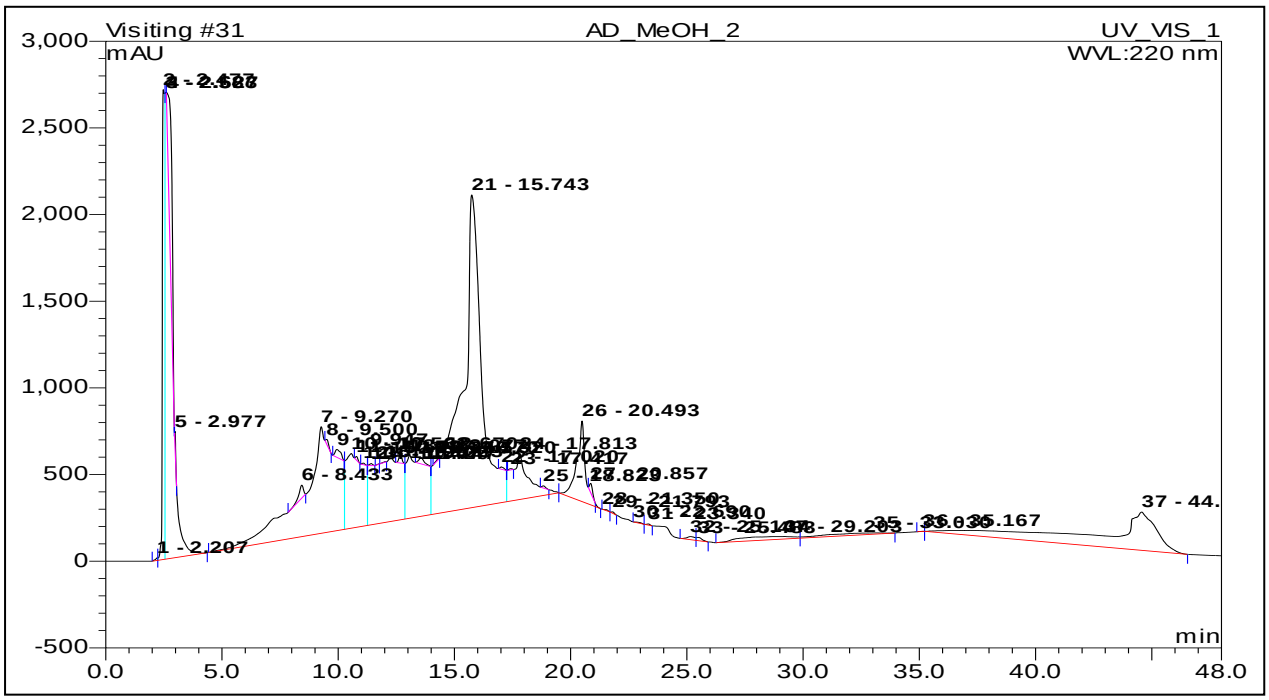


Figure 68: HPLC chromatogram for the SPE fraction obtained from the leaves of *A. digitata*

Table 20: **The retention times, peak heights, areas, relative areas and types of the peaks obtained from HPLC – PDA chromatogram for SPE fraction of *A. digitata***

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel. Area %	Amount
1	2.21	n.a.	14.388	1.781	0.03	n.a.
2	2.48	n.a.	2710.598	332.903	4.87	n.a.
3	2.58	n.a.	2692.104	875.394	12.80	n.a.
4	2.63	n.a.	114.797	178.978	2.62	n.a.
5	2.98	n.a.	70.610	3.997	0.06	n.a.
6	8.43	n.a.	74.655	17.595	0.26	n.a.
7	9.27	n.a.	614.837	1043.656	15.26	n.a.
8	9.50	n.a.	26.740	4.274	0.06	n.a.
9	9.95	n.a.	45.084	13.444	0.20	n.a.
10	10.56	n.a.	430.344	382.267	5.59	n.a.
11	10.79	n.a.	20.558	2.492	0.04	n.a.
12	11.09	n.a.	9.324	1.345	0.02	n.a.
13	11.42	n.a.	12.085	1.887	0.03	n.a.
14	11.75	n.a.	1.854	0.235	0.00	n.a.
15	12.02	n.a.	3.049	0.544	0.01	n.a.
16	12.25	n.a.	365.403	550.011	8.04	n.a.
17	12.67	n.a.	50.782	7.630	0.11	n.a.
18	13.08	n.a.	362.159	349.521	5.11	n.a.
19	13.57	n.a.	35.881	9.697	0.14	n.a.
20	14.32	n.a.	7.154	1.451	0.02	n.a.
21	15.74	n.a.	1804.114	1870.055	27.34	n.a.
22	17.02	n.a.	13.427	2.561	0.04	n.a.
23	17.42	n.a.	7.266	1.108	0.02	n.a.
24	17.81	n.a.	260.737	230.911	3.38	n.a.
25	18.82	n.a.	12.899	2.298	0.03	n.a.
26	20.49	n.a.	465.247	200.811	2.94	n.a.
27	20.86	n.a.	52.172	8.152	0.12	n.a.
28	21.35	n.a.	0.002	0.925	0.01	n.a.
29	21.79	n.a.	7.300	1.140	0.02	n.a.
30	22.69	n.a.	0.005	1.195	0.02	n.a.
31	23.34	n.a.	6.504	1.066	0.02	n.a.
32	25.15	n.a.	17.816	7.276	0.11	n.a.
33	25.48	n.a.	18.179	5.229	0.08	n.a.
34	29.20	n.a.	14.290	49.744	0.73	n.a.
35	33.03	n.a.	7.377	32.379	0.47	n.a.
36	35.17	n.a.	0.698	0.157	0.00	n.a.
37	44.57	n.a.	220.672	645.470	9.44	n.a.
Total			10571.11	6839.579	100.00	0.000

n. a. = not applicable

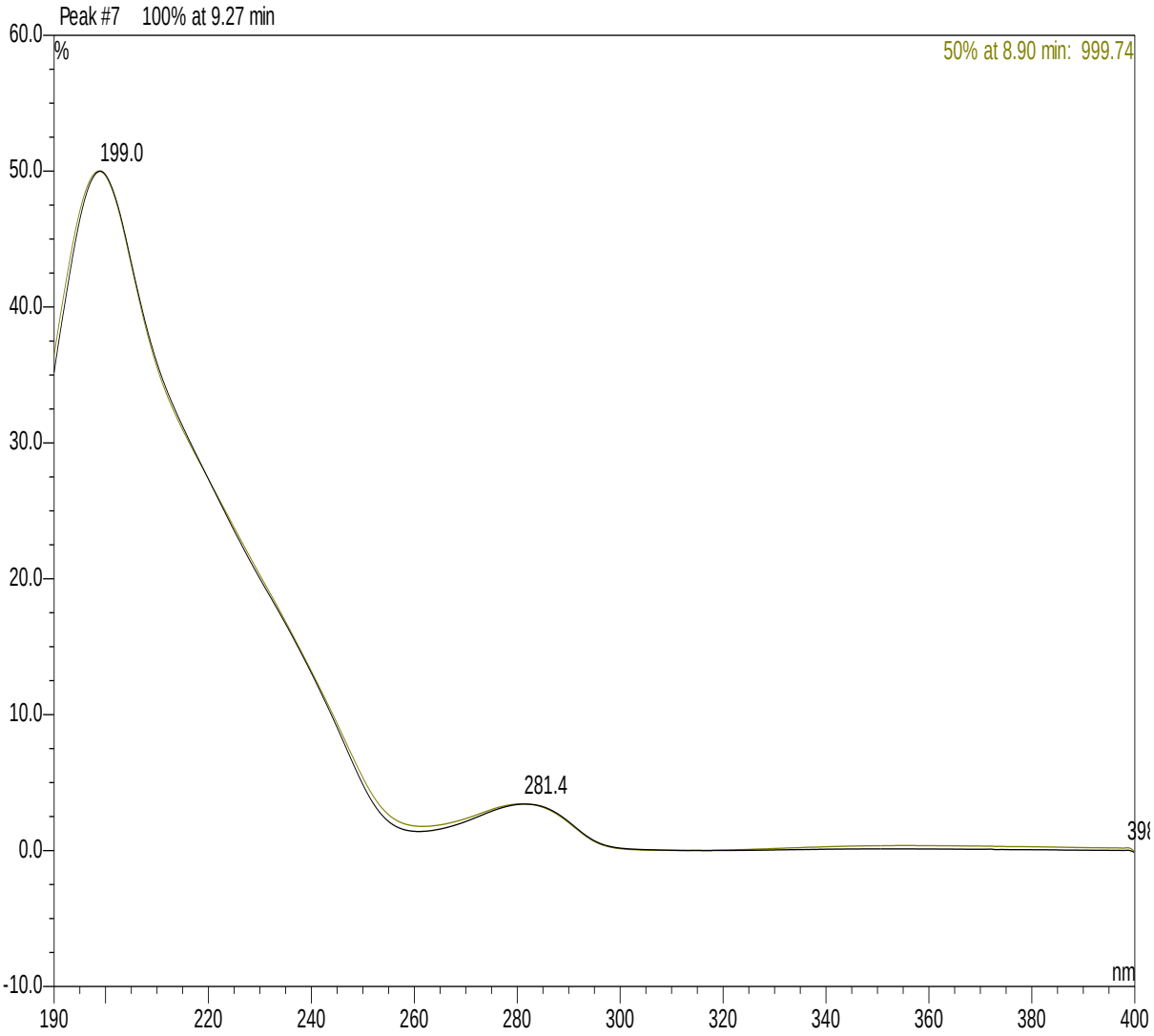


Figure 69: UV-Vis spectrum of the peak from HPLC chromatogram of *A. digitata* in Figure 55 at R_T 9.27 min

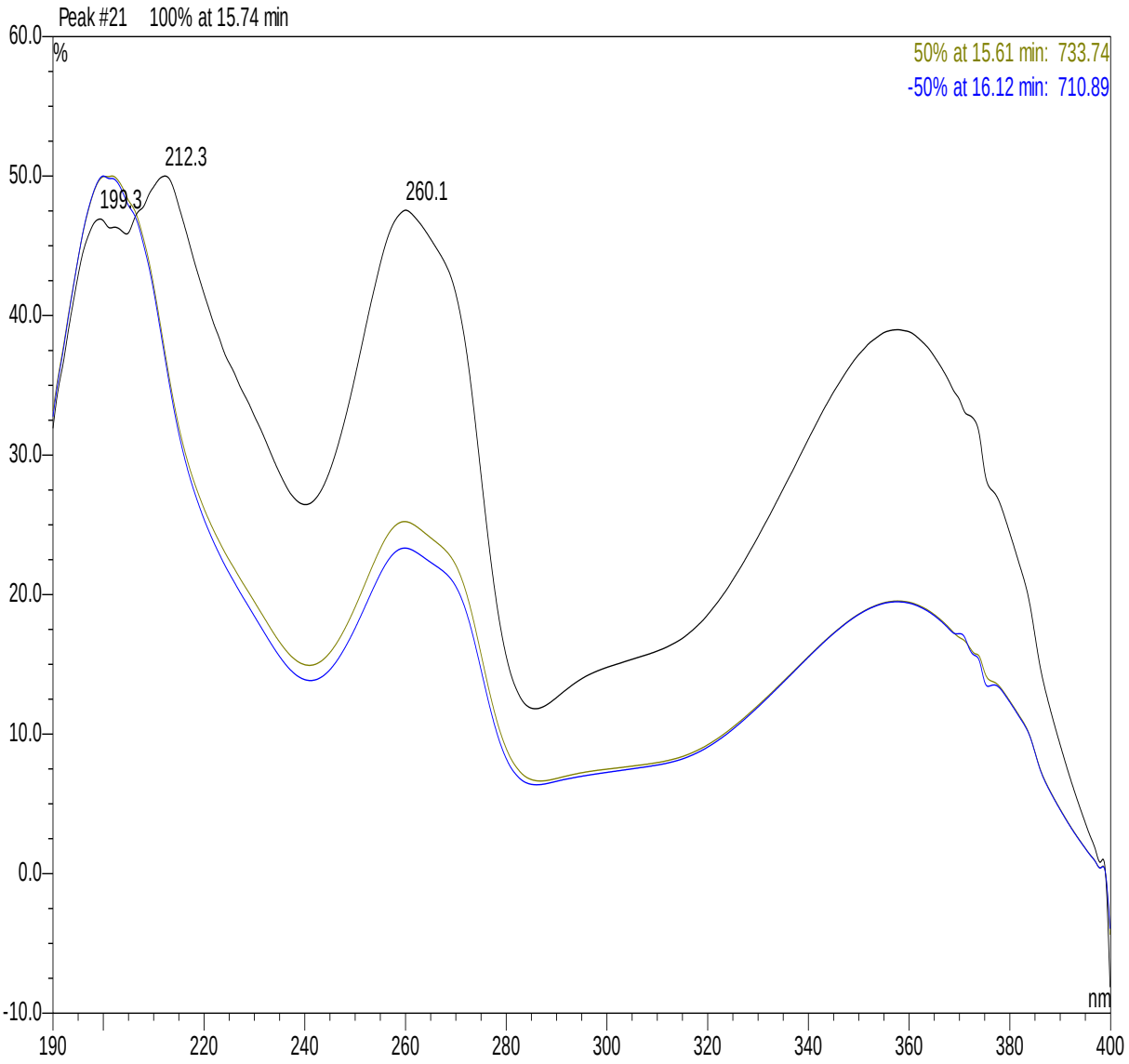


Figure 70: UV-Vis spectrum of the peak from HPLC chromatogram of *A. digitata* in Figure 55 at R_T 15.74 min

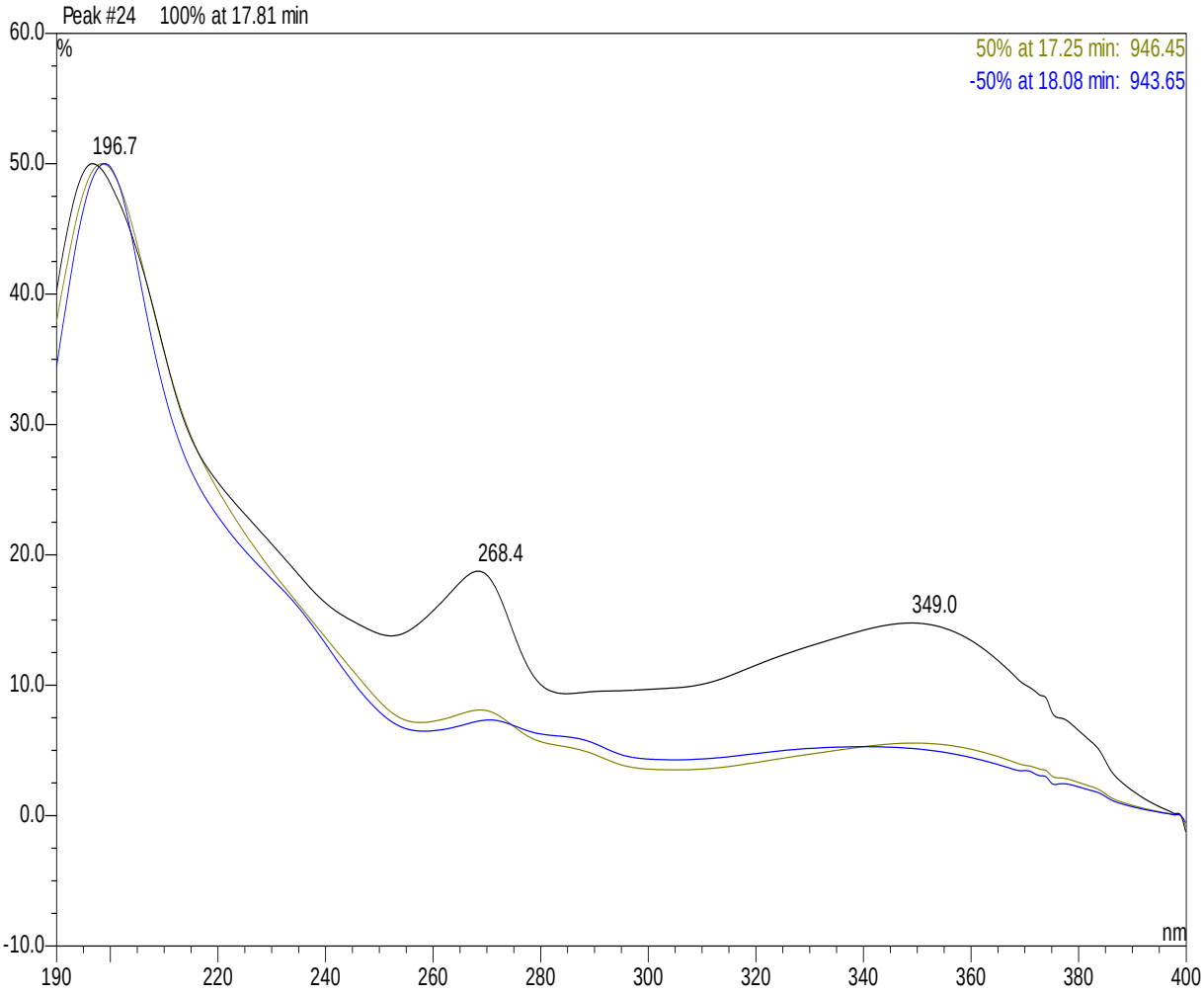


Figure 71: UV-Vis spectrum of the peak from HPLC chromatogram of *A. digitata* in Figure 55 at R_T 17.81min

4.5.2 HPLC analyses of the SPE fractions obtained from the leaves of *C. procera*

The HPLC chromatogram for the SPE fraction obtained from the leaves of *C. procera* is presented in Figure 72. Major peaks were observed at retention times 2.39, 3.21, 6.69, 14.22 and 14.88 minutes. These with the peak heights, areas, relative areas and types of the peaks obtained are shown in Table 21 while the representative UV-Vis spectra showing different absorption maxima from the scanned peaks are presented in Figure 73 - 75.

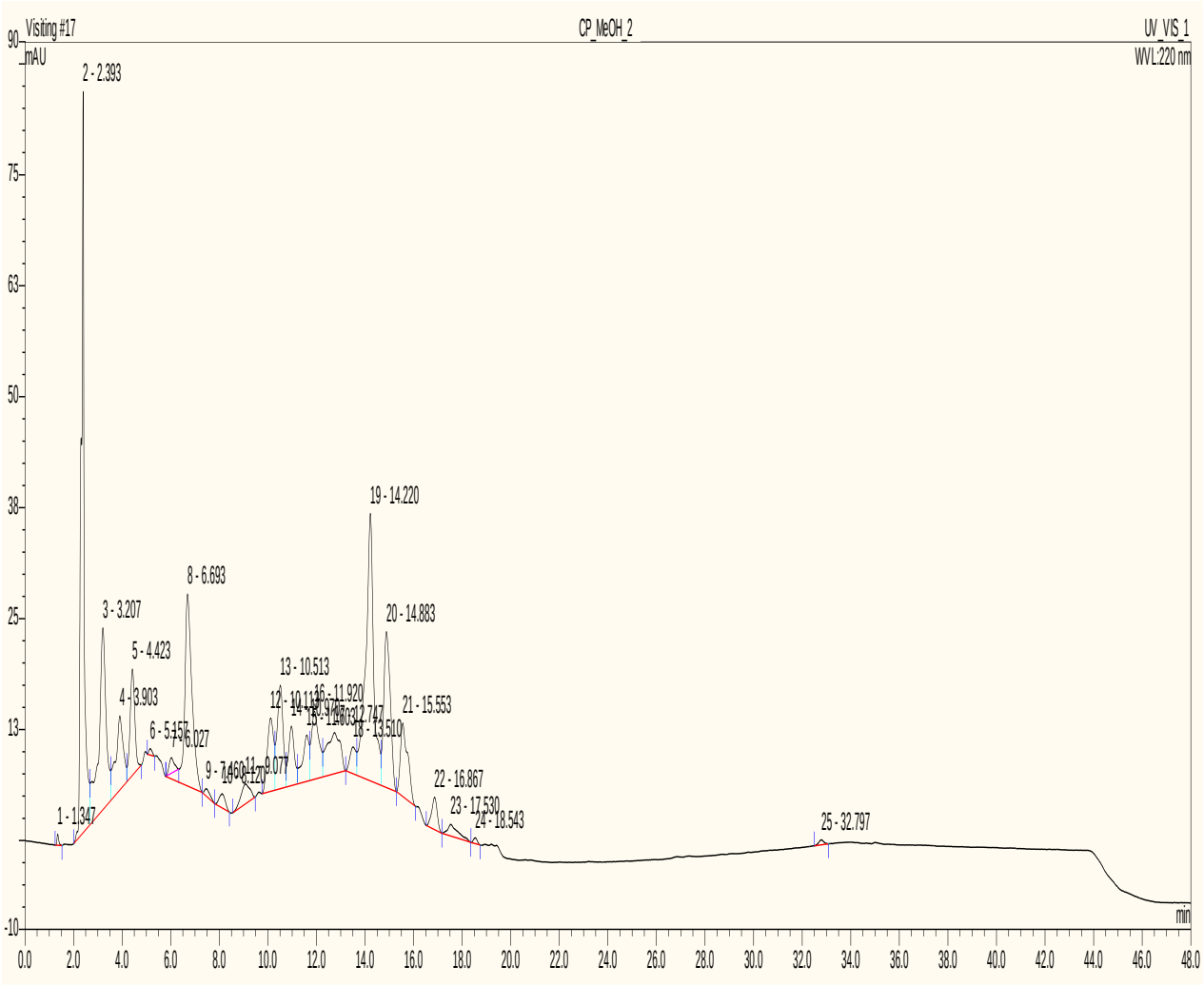


Figure 72: HPLC chromatogram for the SPE fraction obtained from the leaves of *C. procera*

Table 21: The retention times, peak heights, areas and relative areas of the peaks obtained from HPLC – PDA chromatogram for SPE fraction of *C. procera*

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount
1	1.35	n.a.	1.241	0.101	0.14	n.a.
2	2.39	n.a.	83.491	11.830	16.29	n.a.
3	3.21	n.a.	20.482	7.266	10.00	n.a.
4	3.90	n.a.	8.331	3.093	4.26	n.a.
5	4.42	n.a.	11.961	2.736	3.77	n.a.
6	5.16	n.a.	0.705	0.109	0.15	n.a.
7	6.03	n.a.	1.741	0.430	0.59	n.a.
8	6.69	n.a.	21.620	7.678	10.57	n.a.
9	7.46	n.a.	0.804	0.216	0.30	n.a.
10	8.12	n.a.	1.583	0.463	0.64	n.a.
11	9.08	n.a.	2.233	0.952	1.31	n.a.
12	10.11	n.a.	8.251	2.498	3.44	n.a.
13	10.51	n.a.	11.638	3.377	4.65	n.a.
14	10.97	n.a.	6.678	1.898	2.61	n.a.
15	11.60	n.a.	5.201	1.631	2.24	n.a.
16	11.92	n.a.	7.654	2.779	3.83	n.a.
17	12.75	n.a.	4.627	3.014	4.15	n.a.
18	13.51	n.a.	3.004	0.880	1.21	n.a.
19	14.22	n.a.	30.135	11.374	15.66	n.a.
20	14.88	n.a.	17.556	5.544	7.63	n.a.
21	15.55	n.a.	8.251	2.887	3.98	n.a.
22	16.87	n.a.	3.630	0.957	1.32	n.a.
23	17.53	n.a.	1.310	0.671	0.92	n.a.
24	18.54	n.a.	0.650	0.117	0.16	n.a.
25	32.80	n.a.	0.555	0.137	0.19	n.a.
Total:			263.331	72.636	100.00	0.000

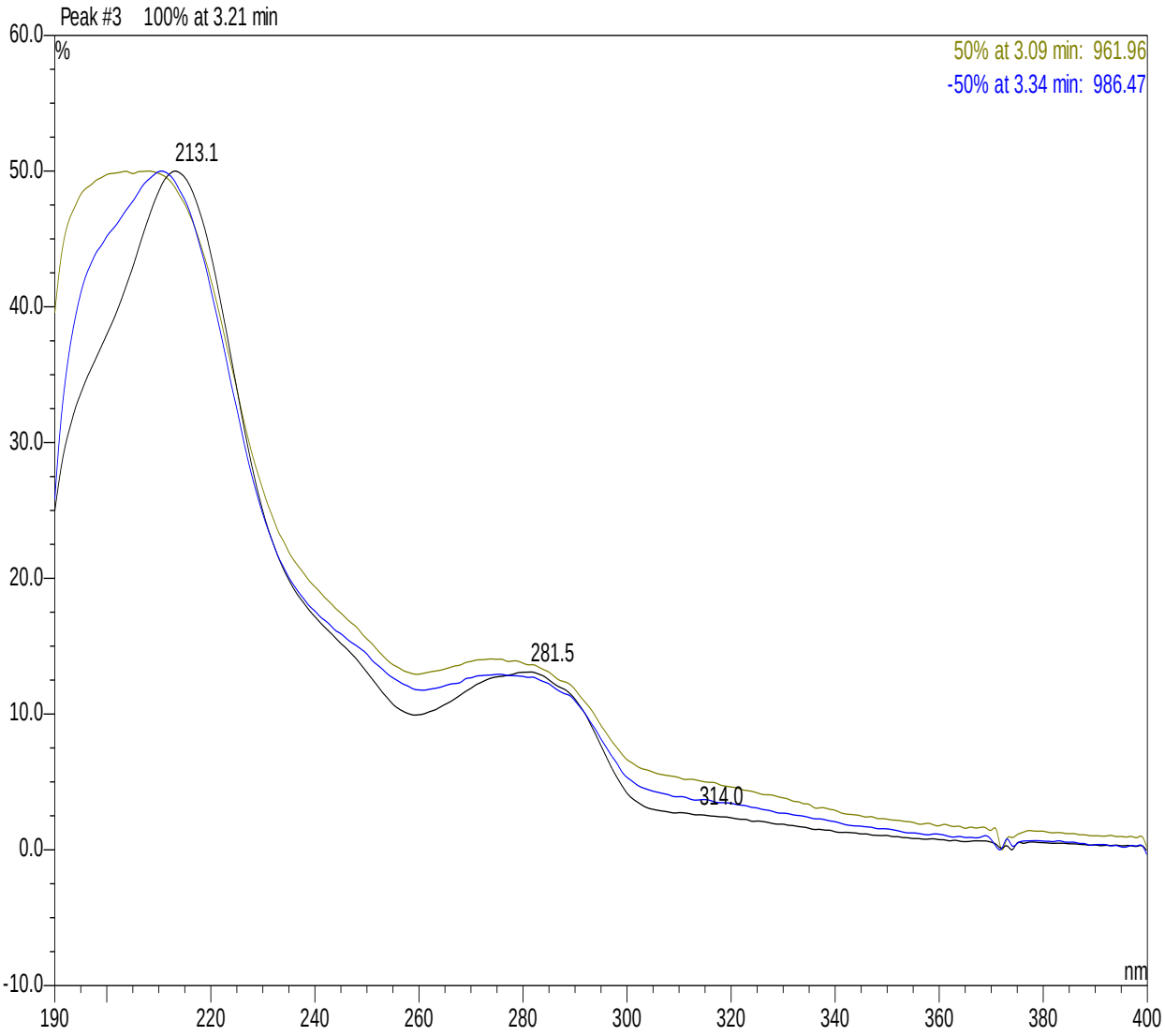


Figure 73: UV-Vis spectrum of the peak from HPLC chromatogram of *C. procera* in Figure 59 at R_T 3.20 min

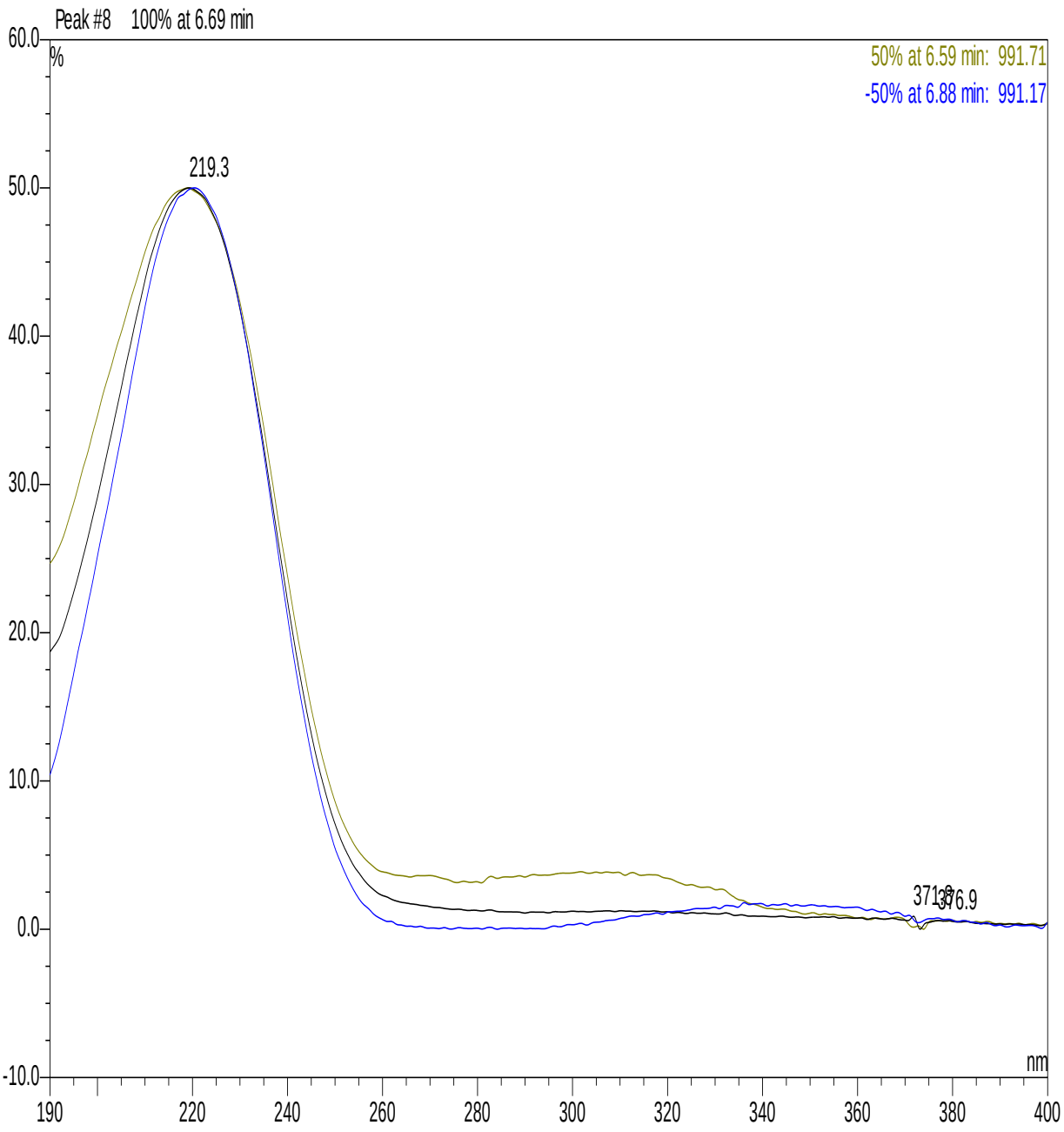


Figure 74: UV-Vis spectrum of the peak from HPLC chromatogram of *C. procera* in Figure 59 at R_T 6.69 min

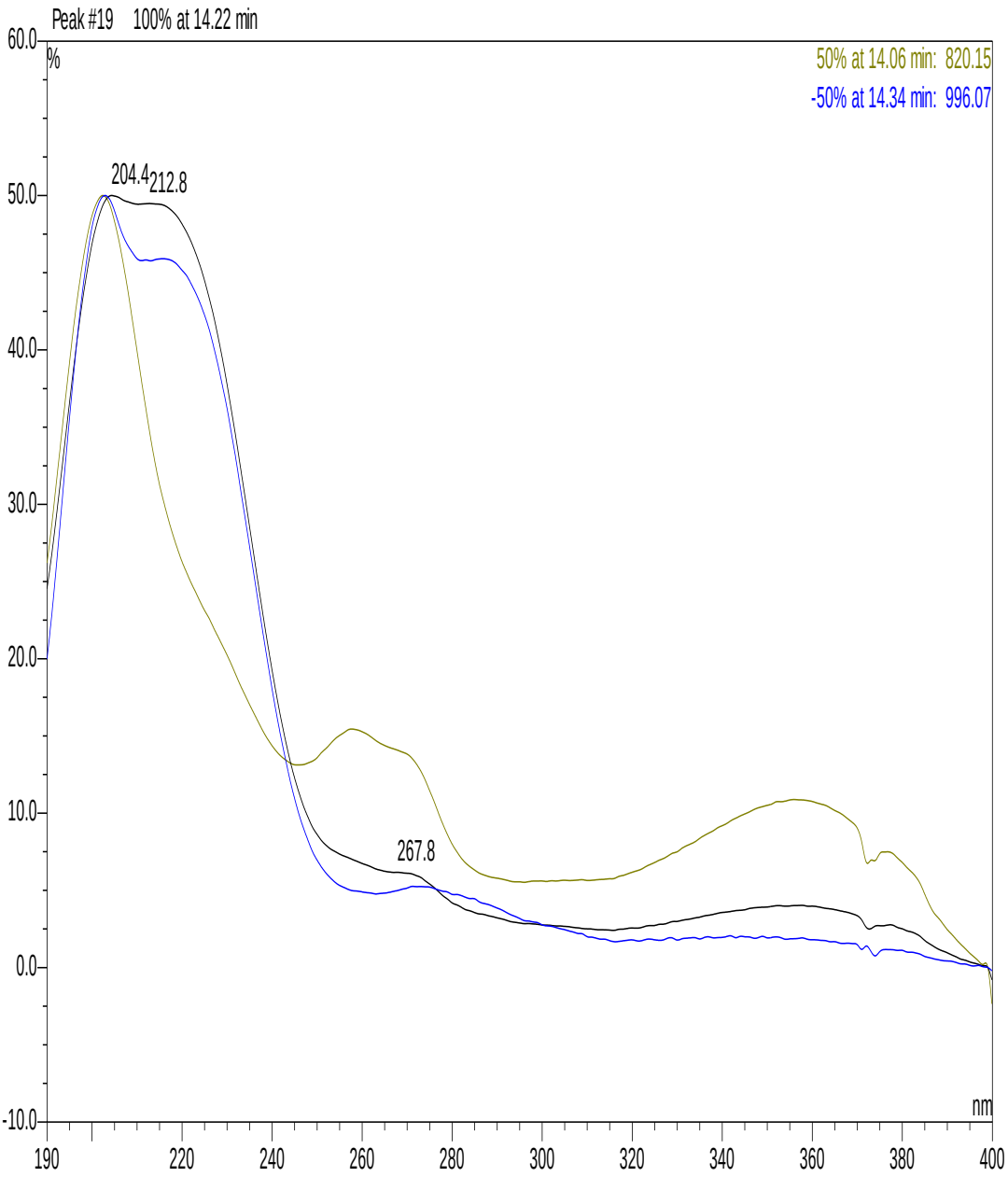


Figure 75: UV-Vis spectrum of the peak from HPLC chromatogram of *C. procera* in Figure 59 at R_T 14.22 min

4.5.3 Results of HPLC analyses of the SPE fractions obtained from the leaves of *E. hirta*

The HPLC chromatogram for the SPE fraction obtained from the leaves of *C. procera* is presented in Figure 76. Major peaks were observed at retention times 2.41, 9.65, 11.03, 11.61, 13.62 minutes. These with the peak heights, areas, relative areas and types of the peaks obtained are shown in Table 22 while the representative uv-vis spectra showing different absorption maxima from the scanned peaks are presented in Figure 77 - 79.

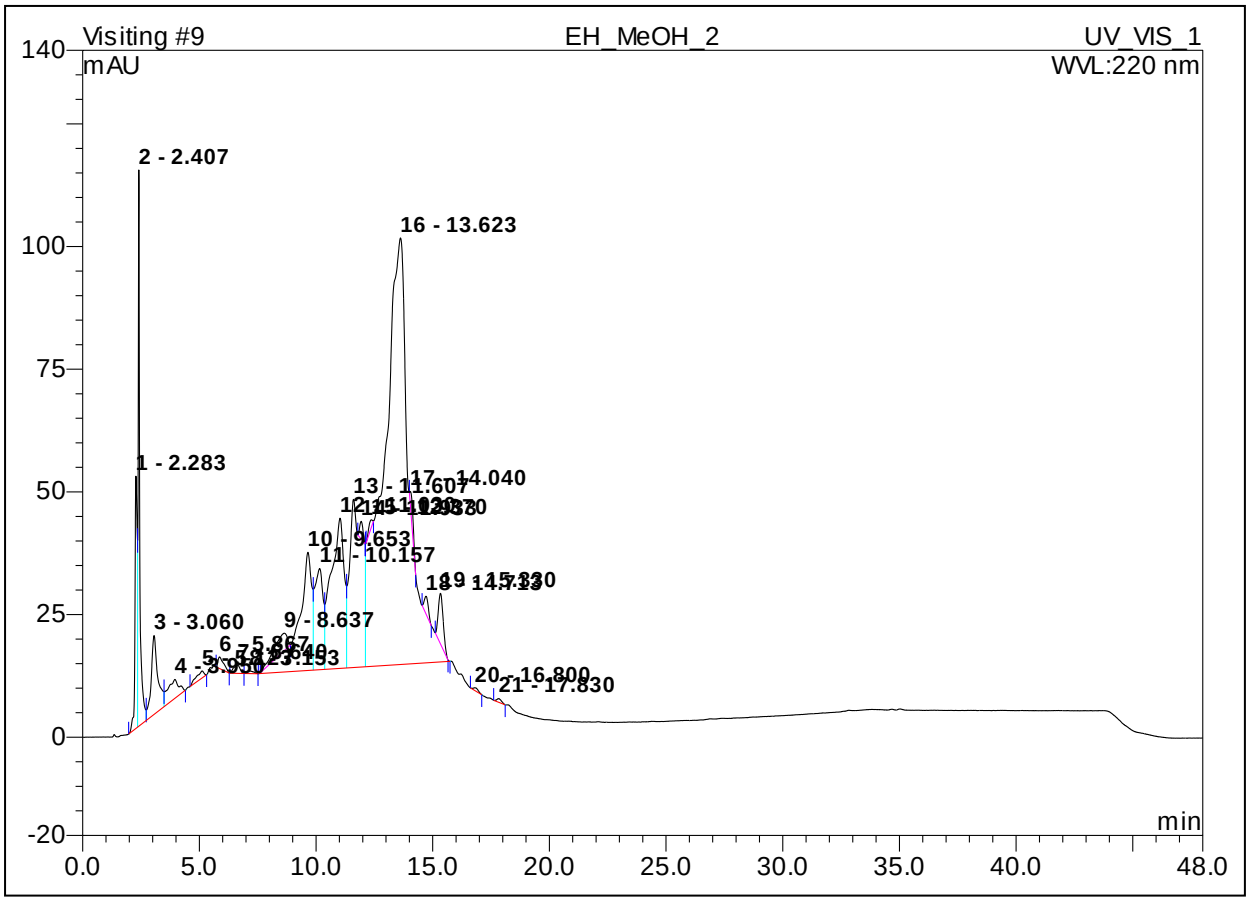


Figure 76: HPLC chromatogram for the SPE fraction obtained from the leaves of *E. hitra*

Table 22: The retention times, peak heights, areas and relative areas of the peaks obtained from HPLC – PDA chromatogram for SPE fraction of *E. hirta*

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel. Area %	Amount
1	2.28	n.a.	51.415	5.630	2.66	n.a.
2	2.41	n.a.	113.350	9.301	4.39	n.a.
3	3.06	n.a.	16.069	5.383	2.54	n.a.
4	3.95	n.a.	3.851	2.272	1.07	n.a.
5	5.12	n.a.	1.389	0.592	0.28	n.a.
6	5.87	n.a.	2.370	0.679	0.32	n.a.
7	6.64	n.a.	1.670	0.525	0.25	n.a.
8	7.15	n.a.	0.529	0.172	0.08	n.a.
9	8.64	n.a.	3.573	2.508	1.18	n.a.
10	9.65	n.a.	24.086	17.098	8.08	n.a.
11	10.16	n.a.	20.625	8.664	4.09	n.a.
12	11.03	n.a.	30.609	20.271	9.58	n.a.
13	11.61	n.a.	34.285	21.211	10.02	n.a.
14	11.93	n.a.	3.596	0.577	0.27	n.a.
15	12.37	n.a.	1.524	0.327	0.15	n.a.
16	13.62	n.a.	86.930	112.104	52.97	n.a.
17	14.04	n.a.	2.593	0.741	0.35	n.a.
18	14.71	n.a.	3.608	0.731	0.35	n.a.
19	15.33	n.a.	10.284	2.514	1.19	n.a.
20	16.80	n.a.	0.551	0.152	0.07	n.a.
21	17.83	n.a.	0.743	0.175	0.08	n.a.
Total			413.648	211.629	100.00	0.000

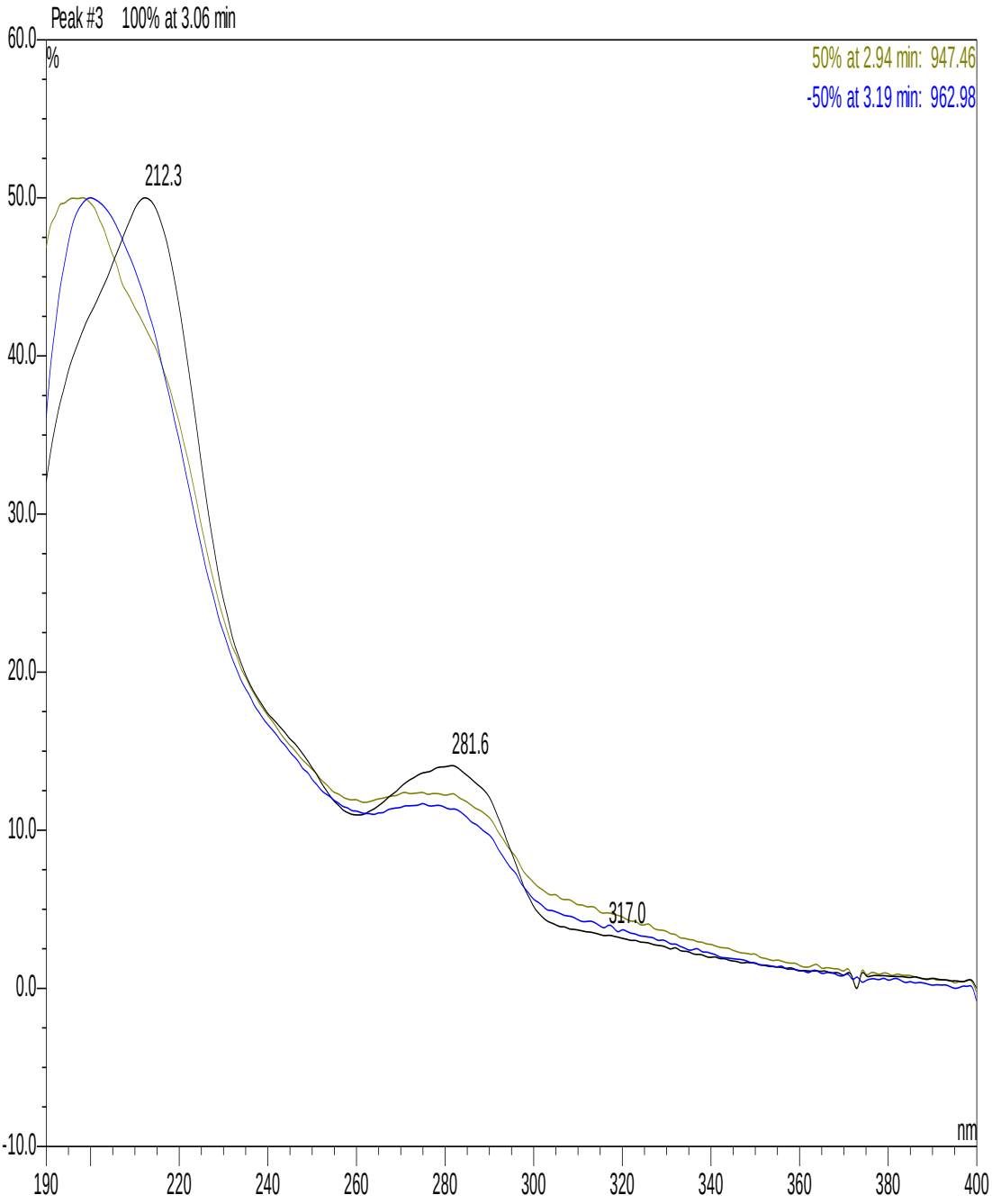


Figure 77: UV-Vis spectrum of the peak from HPLC chromatogram of *E. hirta* in Figure 63 at R_T 3.06 min

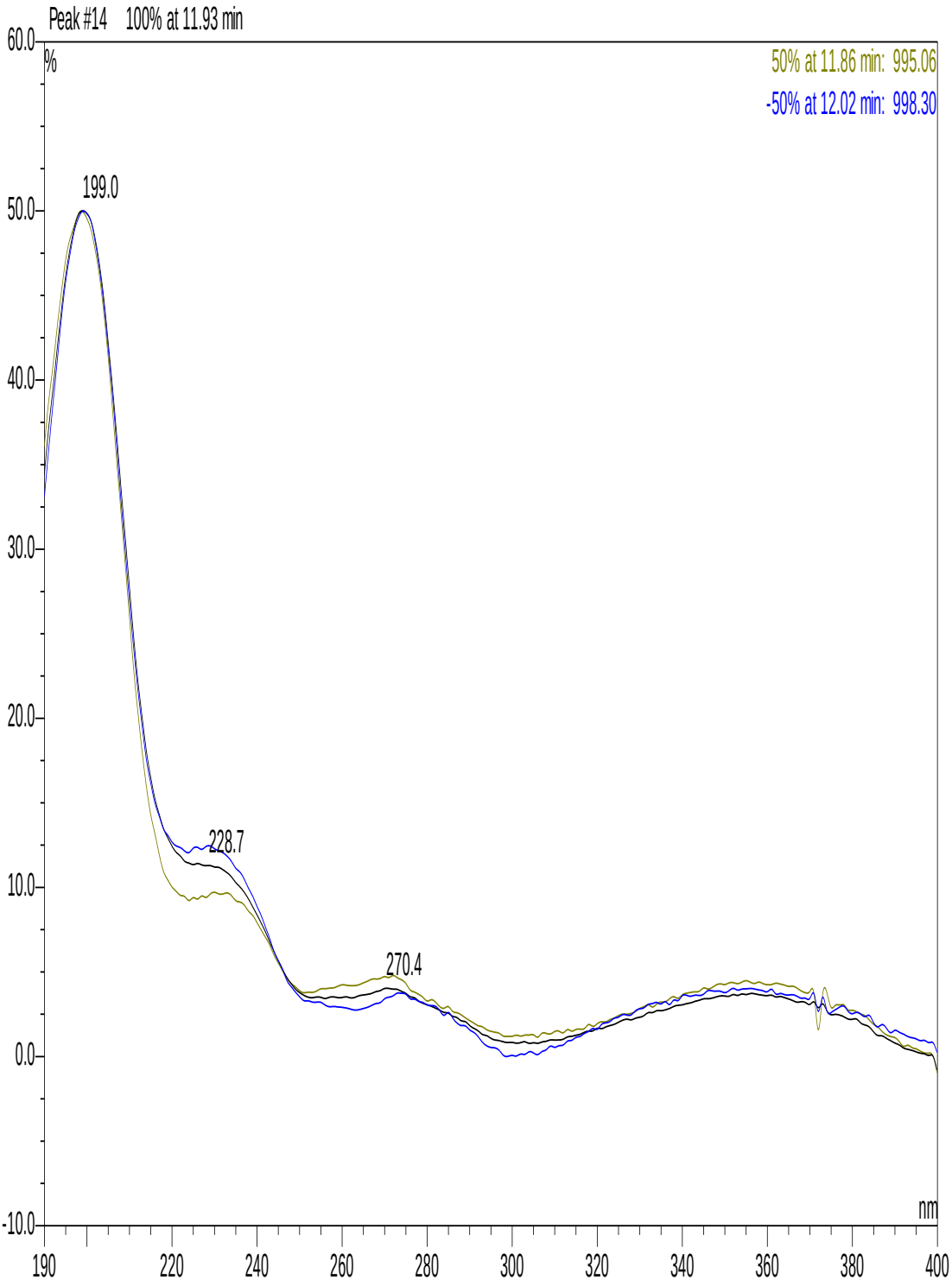


Figure 78: UV-Vis spectrum of the peak from HPLC chromatogram of *E. hirta* in Figure 63 at R_T 11.93 min

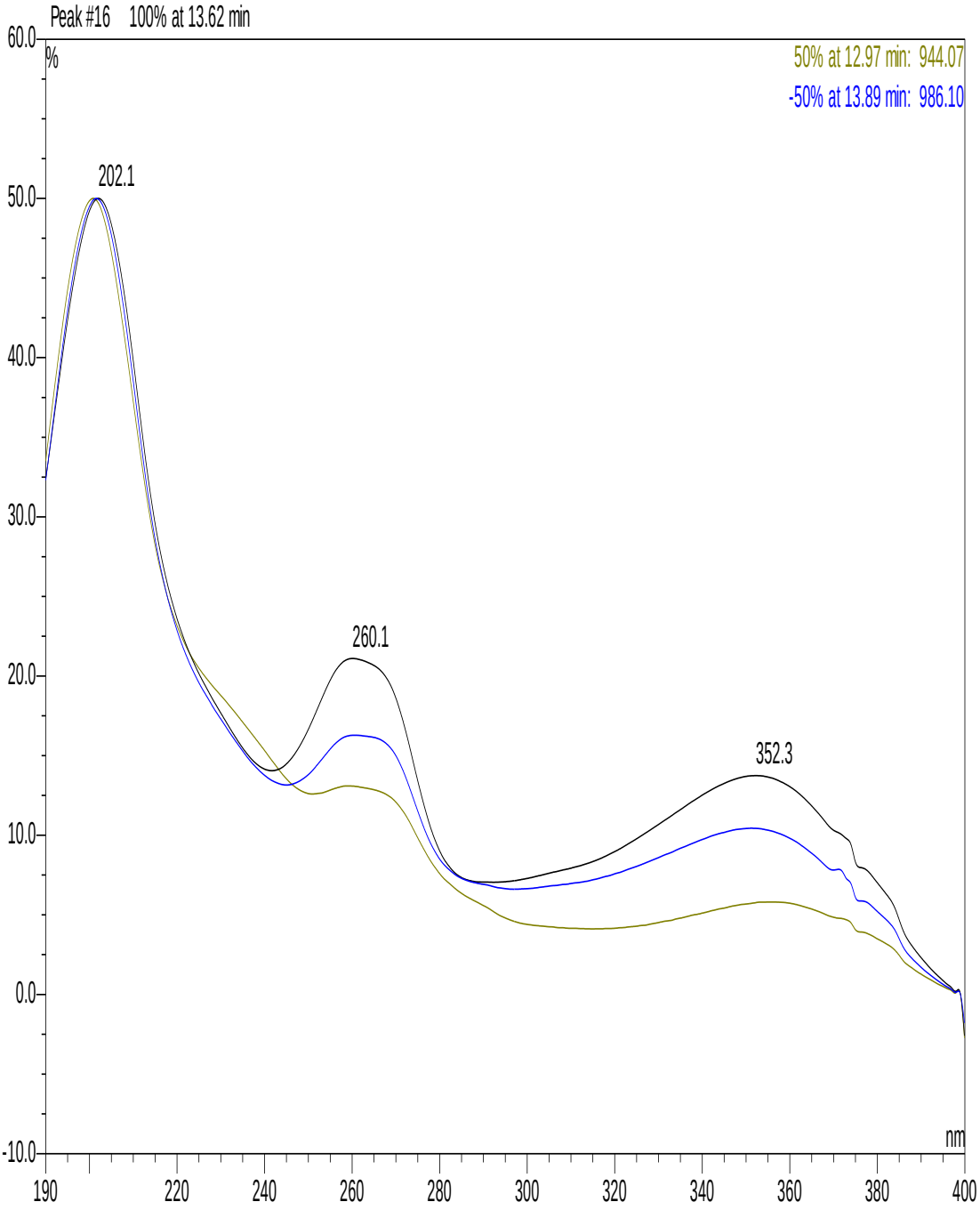


Figure 79: UV-Vis spectrum of the peak from HPLC chromatogram of *E. hirta* in Figure 63 at R_T 13.62 min

4.6 Bioctivity-guided isolation of an antioxidant from ethyl acetate fraction of *A. digitata* [AD(EtOAc)] and mass spectral data

The ethyl acetate fraction of *A. digitata* exhibited highest antioxidant activity among the extracts and fractions obtained using different solvents hence was used for the isolation. A prominent peak was obtained from the HPLC chromatographic analyses of the ethyl acetate fraction of *A. digitata* (Figure 80). The peak obtained at R_T 20.110 min was scanned and the UV-Vis spectrum (Figure 81) showed absorption maxima at 200.5, 258.4 and 374.7 nm. The constituent was collected and further chromatographed and on elution appeared as a huge peak at R_T 19.367 minutes (Figure 82). Quercetin standard was eluted at R_T of 19.522 minutes under the same conditions (Figure 83). The constituent was dried and a crystalline yellow powder with melting point of 314.5^{o}C was obtained and the mass spectrum had a base peak m/z value of 301.035 (Figure 84). Figure 85 shows the observed data as compared with the theoretical isotope (M+H). The mass spectral data gave the molecular peak of the isolated compound with m/z 301.0351 and that of the standard quercetin, m/z 301.0354 (Figure 85).

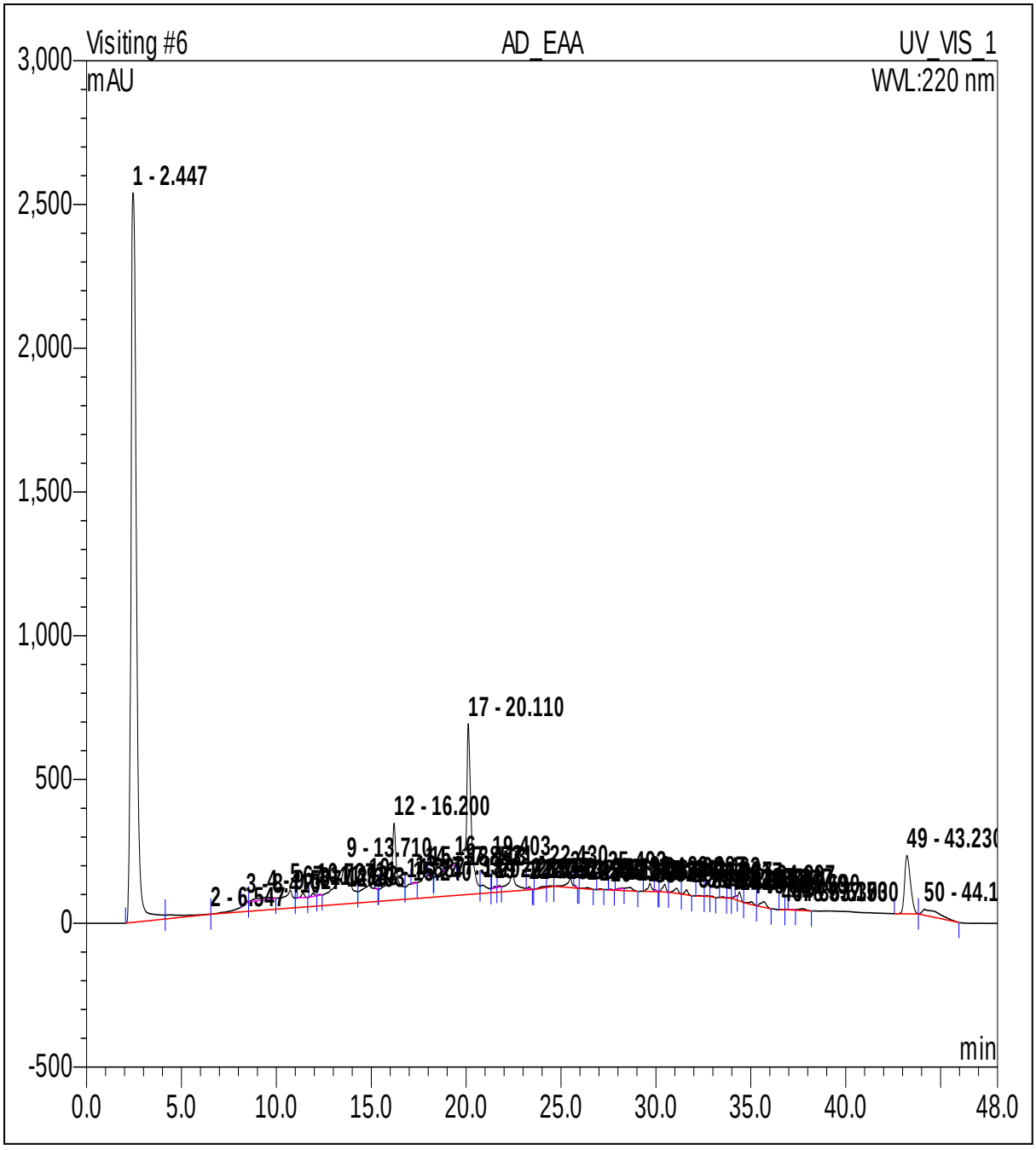


Figure 80: HPLC Chromatogram of ethyl acetate fraction obtained from the leaves of *A. digitata*

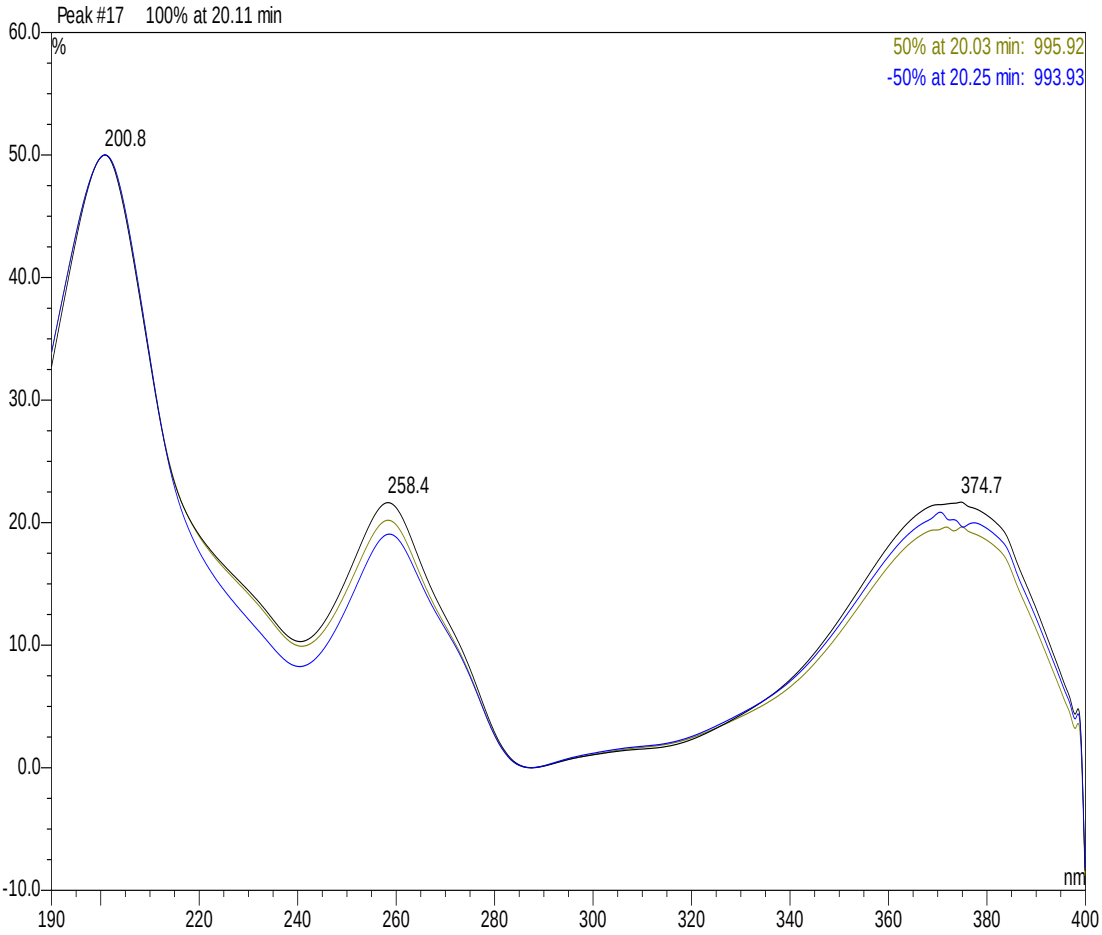


Figure 81: UV-Vis spectrum of the peak from the HPLC chromatogram of ethyl acetate fraction of *A. digitata* in Figure 68 at R_T 20.110 min

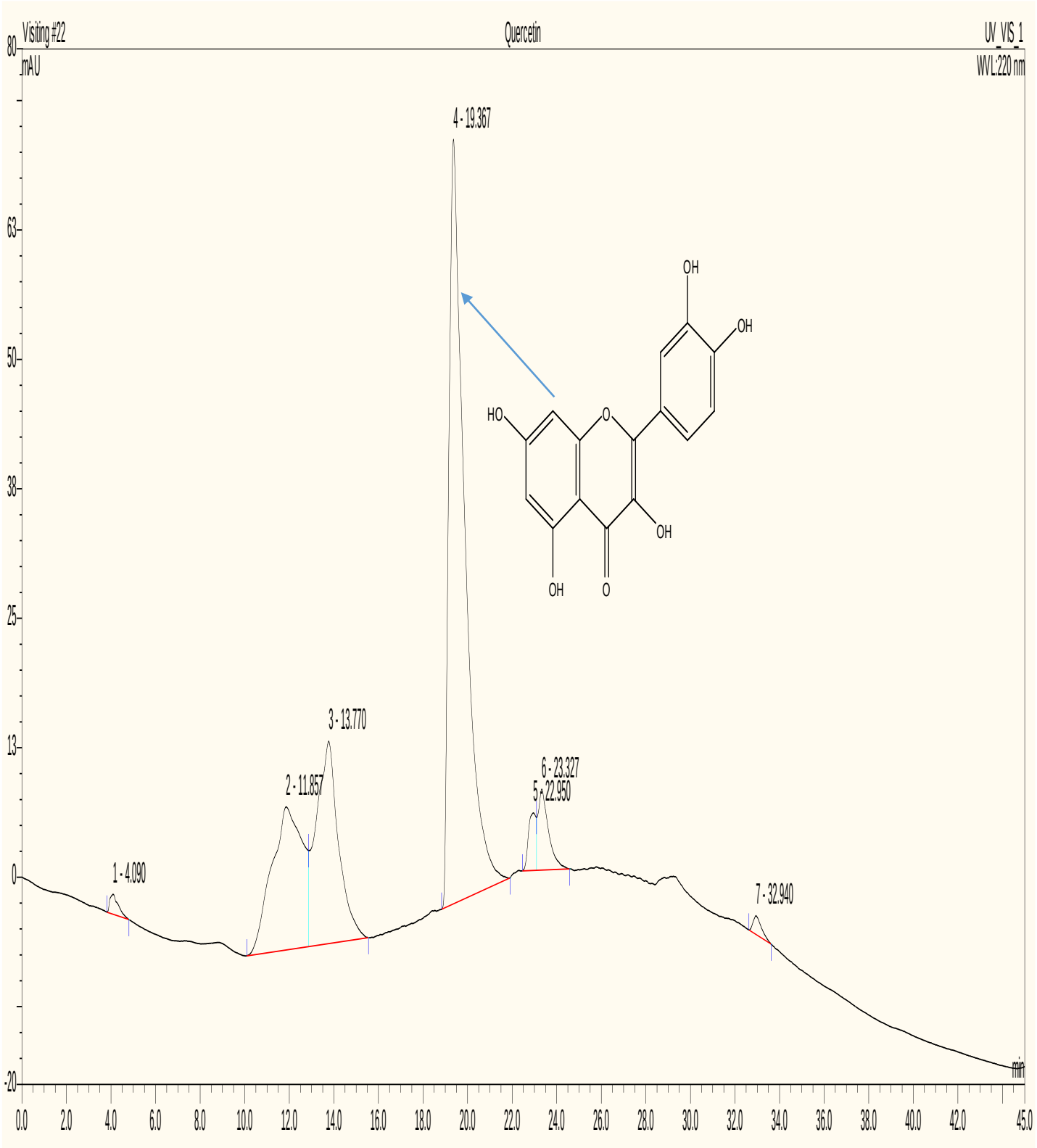
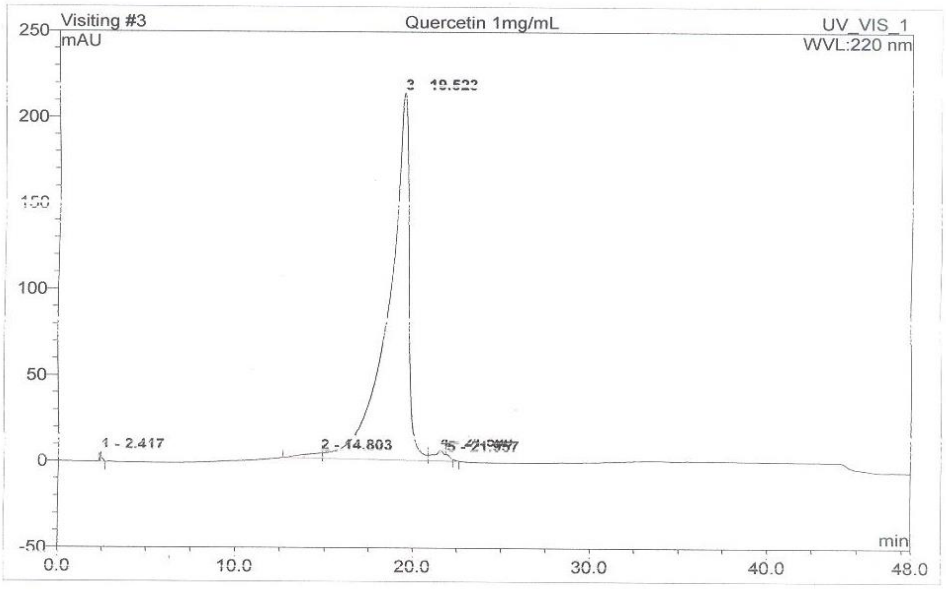


Figure 82: HPLC Chromatogram of quercetin isolated from ethyl acetate fraction of *A. digitata* at R_T of 19.367 mins

3 Quercetin 1mg/mL

Sample Name:	**Quercetin 1mg/mL**	*Injection Volume:*	**400.0**
Vial Number:	**RA1**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**220.0**
Control Program:	**20100Grad_3ml_Semiprep**	*Bandwidth:*	**1**
Quantif. Method:	**Column Compartment test 070509**	*Dilution Factor:*	**1.0000**
Recording Time:	**7/9/2012 11:21**	*Sample Weight:*	**1.0000**
Run Time (min):	**48.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2.42	n.a.	5.463	0.590	0.20	n.a.	BMB
2	14.80	n.a.	3.135	3.847	1.31	n.a.	BM
3	19.52	n.a.	213.488	282.766	96.60	n.a.	M
4	21.59	n.a.	6.618	5.382	1.84	n.a.	MB
5	21.96	n.a.	0.593	0.141	0.05	n.a.	Rd
Total:			229.297	292.726	100.00	0.000	

Figure 83: HPLC Chromatogram of quercetin standard at retention time of 19.522 minutes

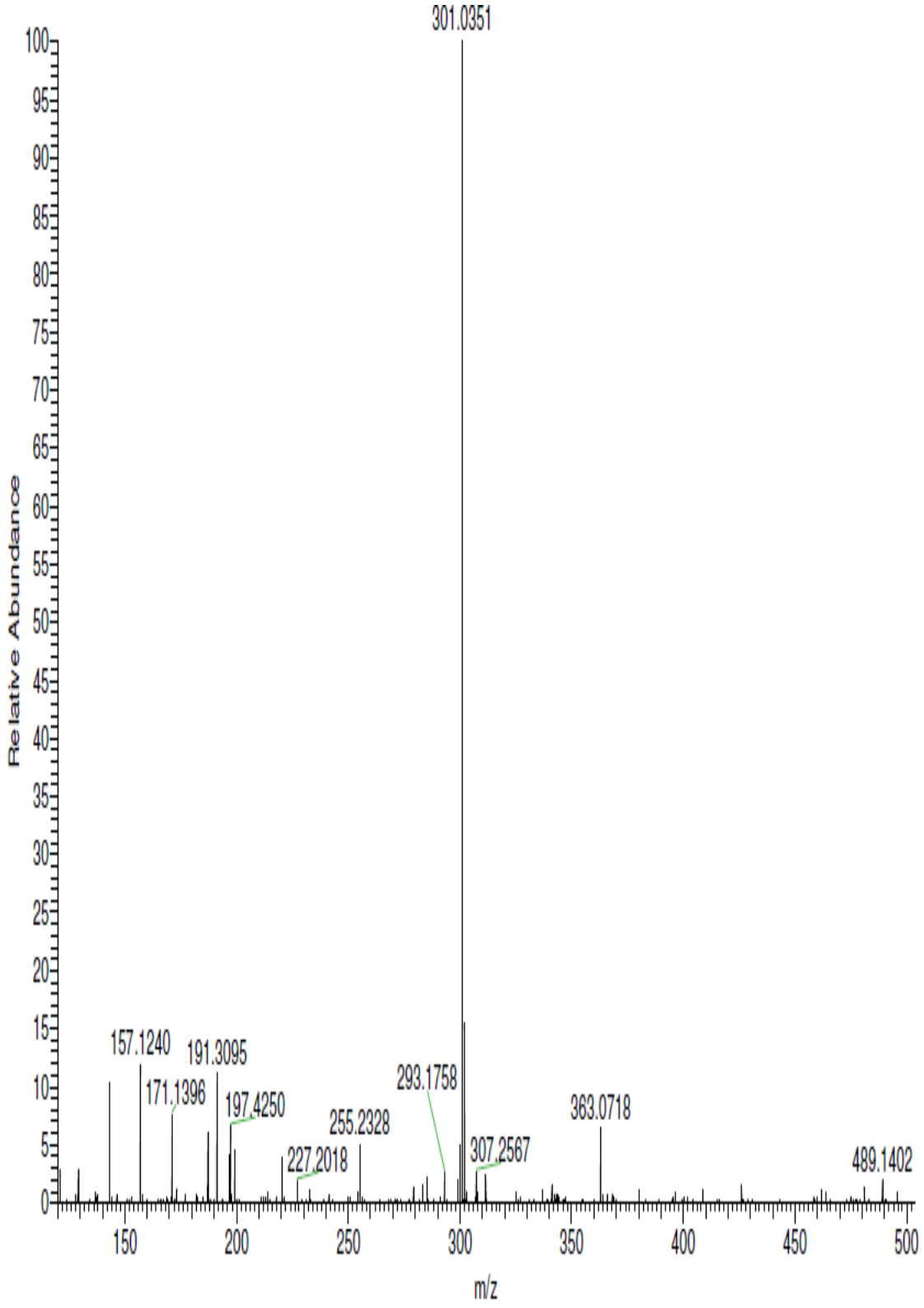


Figure 84: Mass spectrum of the isolated Quercetin showing the molecular peak at m/e 301.0351

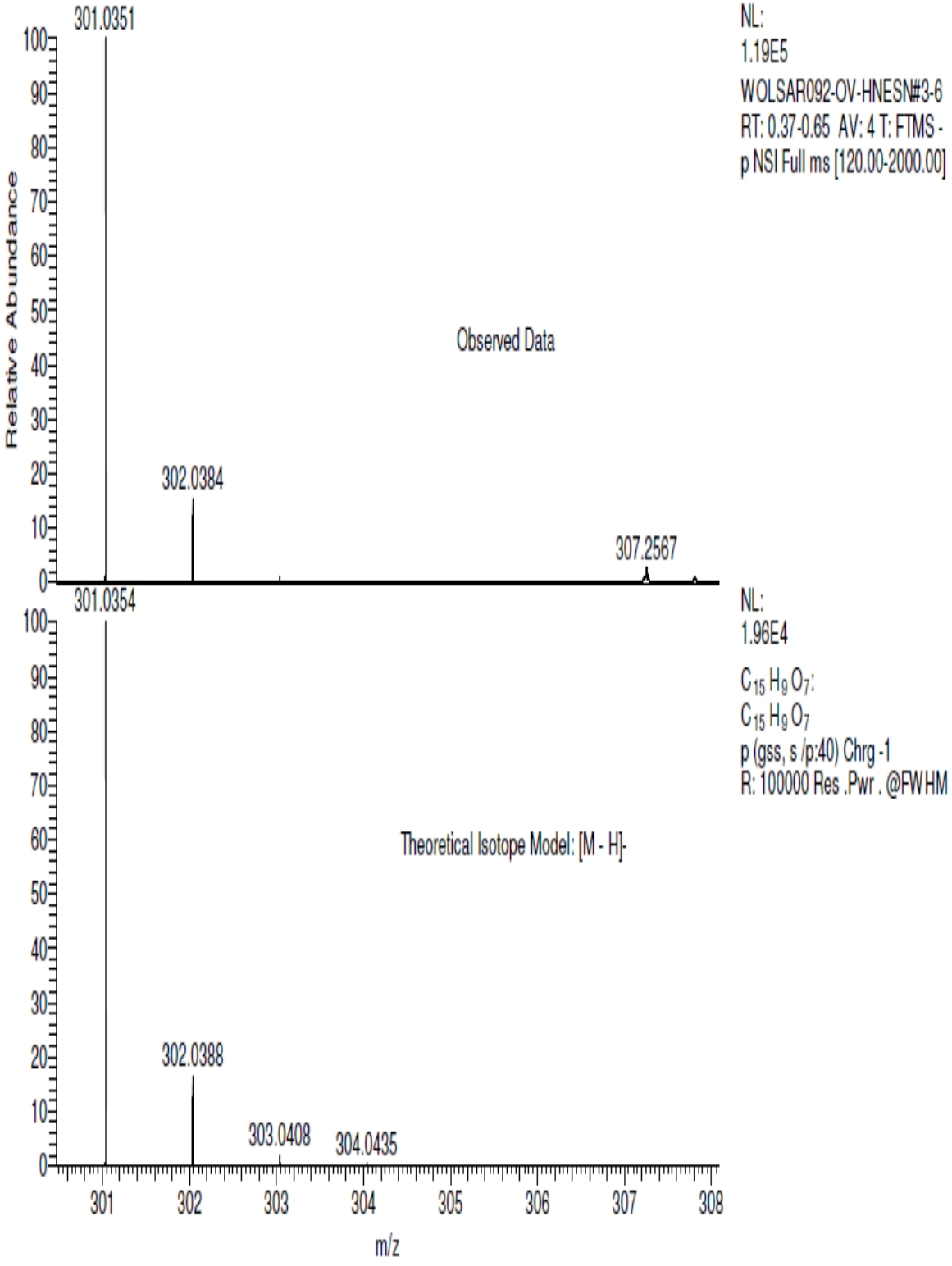


Figure 85: Mass spectra comparing the observed data with the theoretical isotope model (M+H)

4.7 The *in vitro* Biossays

4.7.1 Antioxidant activities of all the extracts and fractions from *Adansonia digitata* (AD), *Calotropis procera* (CP) and *Euphorbia hirta* (EH)

The antioxidant activity of an extract is indicated by its percentage inhibition of free radicals and 50% Inhibition concentration. Percentage inhibition was determined for various extracts and fractions from 50% aqueous methanol crude extracts and their hexane, ethylacetate and butanol fractions; soxhlet hexane, dichloromethane and methanol extracts and methanolic solid phase extraction (SPE) fractions obtained using 20%, 40% 60%, 80% aq. MeOH and 100% MeOH of *Adansonia digitata* leaves (AD), *Calotropis procera* leaves (CP) and *Euphorbia hirta* aerial parts (EH). The results are presented in Table 23 and plots of percentage inhibition (%I) against different concentrations (0.0001 – 1 mg/mL) of the extracts and fractions in comparison to quercetin standard at the same concentrations are represented in Figures 86 - 95.

The inhibiting abilities of different extracts and fractions of the plant samples on DPPH were ranked using both the percentage inhibition (%I) and 50% Inhibition Concentrations (IC_{50}). An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} values for different extracts and fractions of AD, CP and EH are shown in Table 24. The lower the $IC_{50,}$ the higher the antioxidant activity. It was observed that the crude extracts of AD and EH obtained from the 50% aq.MeOH extraction (1 mg/mL) had moderate percentage inhibition of 72.538 ± 0.001% and 66.211 ± 0.021% (Table 23) with IC_{50} of 36.88 x 10^{-2} and 66.03 x 10^{-2} mg/mL (Table 24) respectively as compared to quercetin standard (%I: 95.521± 0.010 %, IC_{50}: 0.52 x 10^{-2} mg/mL) at the same concentration of 1 mg/mL. The crude extract of CP had low %I of 10.567 ± 0.003% at the highest concentration of 1mg/mL indicating no IC_{50} (percentage inhibition was not up to 50%) hence recorded as 'No Activity (NA)' in Table 24. All the extracts obtained using hexane and

dichloromethane (DCM) had no IC_{50}. However, the values of %I obtained for hexane fractions using the 50% aq.MeOH were slightly higher (17.482 ± 0.001, 12.345 ± 0.001 and 49.712 ± 0.005% for AD, CP and EH respectively) than those of soxhlet extracts (12.553 ± 0.008, 8.708 ± 0.008 and 10.762 ± 0.002% for AD, CP and EH respectively) at the maximum concentration of 1mg/mL The ethyl acetate fractions of the three plants showed moderate to good %I at 1 mg/mL in the range of 57.842 ± 0.028 – 89.895 ± 0.001% while they exhibited low activities at lower concentrations of 0.0001 to 0.1 mg/mL. Butanol fractions had appreciable antioxidant activities (%I: 76.160 ± 0.017 – 83.261 ± 0.018%). Hexane, DCM and MeOH were used for soxhlet extractions of the 3 plants but only the methanol extracts of AD and EH had IC_{50} and recorded high and moderate antioxidant activities. The highest was AD with percentage inhibition of 89.633 ± 0.226 % and IC_{50} of 4.023 $x10^{-2}$ mg/mL. The highest antioxidant activity among both 50% aq. MeOH and soxhlet extracts was observed in the ethyl acetate fraction of AD (%I: 89.895 ± 0.001%, IC_{50} of 3.476 $x10^{-2}$ mg/mL) hence antioxidant activity – guided isolation was carried out using the ethyl acetate fraction of *Adansonia digitata*.

Solid Phase Extraction was carried out on the soxhlet methanol extracts using 20, 40, 60, 80 and 100% MeOH coded as 1,2,3,4, and 5 respectively. The results of the antioxidant assay showed that there was an increase in antioxidant activity of the fractions obtained compared to the methanol extract and the highest activity was recorded by 40 and 60% aq. MeOH fractions in each of the plants. The 40% MeOH of *A. digitata* gave the highest % I of 94.962 ± 0.004% and IC_{50} of 2.93 $x10^{-2}$ mg/mL which is the highest and the % I is comparable to that of quercetin standard (95.521 ± 0.010 %) of the same concentrations (1 mg/mL). These results are shown in Tables 23, 24 and Figure 93.

Table 23: Percentage Inhibition of Extracts and Fractions of AD, CP and EH with quercetin standard at different concentrations

Concentration (mg/mL)	0.0001	0.001	0.01	0.1	1
SAMPLES	**% INHIBITION**				
Quercetin	1.979 ± 0.033	10.729 ± 0.053	93.854 ± 0.002	94.583 ± 0.001	95.521 ± 0.010
50% MeOH crude extracts					
AD(Ex)	1.204 ± 0.016	2.846 ± 0.017	7.734 ± 0.013	68.257± 0.023	72.538 ± 0.001
CP(Ex)	0.111 ± 0.006	0.278 ± 0.008	0.946 ± 0.004	1.613 ± 0.043	10.567 ± 0.003
EH(Ex)	0.168 ± 0.016	1.284 ± 0.016	1.508 ± 0.030	6.816 ± 0.000	66.211 ± 0.021
Fractions from crude extracts					
$AD(Hex)_m$	2.116 ± 0.005	2.376 ± 0.003	3.302 ± 0.014	7.482 ± 0.001	17.482 ± 0.001
AD(EtOAc)	4.181 ± 0.011	7.549 ± 0.113	28.571 ± 0.002	82.811 ± 0.001	89.895 ± 0.001
AD(BuOH)	2.160 ± 0.008	3.132 ± 0.011	11.015 ± 0.021	73.326 ± 0.001	83.261 ± 0.018
$CP(Hex)_m$	1.936 ± 0.066	3.208 ± 0.057	5.642 ± 0.069	6.704 ± 0.006	12.345 ± 0.004
CP(EtOAc)	0.117 ± 0.016	1.152 ± 0.021	2.334 ± 0.008	23.104 ± 0.014	72.345 ± 0.008
CP(BuOH)	0.510 ± 0.005	2.697 ± 0.006	9.709 ± 0.005	15.102 ± 0.088	76.160 ± 0.017
$EH(Hex)_m$	0.438 ± 0.039	2.510 ± 0.057	4.583 ± 0.062	10.529 ± 0.033	49.712 ± 0.005
EH(EtOAc)	1.440 ± 0.010	2.572 ± 0.025	5.453 ± 0.062	57.305 ± 0.002	57.842 ± 0.028
EH(BuOH)	3.513 ± 0.010	4.038 ± 0.013	5.454 ± 0.018	34.137 ± 0.010	78.815 ± 0.014
Extracts from soxhlet extraction					
AD(Hex)	1.733 ± 0.022	2.420 ± 0.005	2.874 ± 0.025	4.412 ± 0.006	12.553 ± 0.008
AD(DCM)	0.839 ± 0.008	1.572 ± 0.009	1.677 ± 0.004	11.845 ± 0.025	27.358 ± 0.032
AD(MeOH)	2.304 ± 0.024	7.539 ± 0.007	10.89 ± 0.008	80.838 ± 0.198	89.633 ± 0.226
CP(Hex)	2.385 ± 0.030	3.549 ± 0.006	4.548 ± 0.002	5.935 ± 0.000	8.708 ± 0.008

CP(DCM)	0.719 ± 0.012	1.327 ± 0.004	3.153 ± 0.025	3.540 ± 0.021	28.539 ± 0.047
CP(MeOH)	0.680 ± 0.039	0.906 ± 0.004	1.529 ± 0.015	8.551 ± 0.001	31.484 ± 0.083
EH(Hex)	1.490 ± 0.011	3.477 ± 0.018	5.877 ± 0.011	5.629 ± 0.021	10.762 ± 0.002
EH(DCM)	0.977 ± 0.011	1.302 ± 0.048	2.170 ± 0.014	2.984 ± 0.001	22.029 ± 0.032
EH(MeOH)	0.742 ± 0.042	1.483 ± 0.045	3.879 ± 0.001	6.845 ± 0.054	69.652 ± 0.001
Fractions from SPE					
AD(MeOH)1	3.050 ± 0.001	3.125 ± 0.012	6.034 ± 0.001	46.444 ± 0.021	88.254 ± 0.000
AD(MeOH)2	4.073 ± 0.004	5.038 ± 0.018	25.723 ± 0.025	87.995 ± 0.013	94.962 ± 0.004
AD(MeOH)3	4.560 ± 0.013	10.791 ± 0.046	17.709 ± 0.017	75.190 ± 0.001	91.835 ± 0.011
AD(MeOH)4	1.292 ± 0.022	1.615 ± 0.030	17.223 ± 0.045	64.801 ± 0.013	90.420 ± 0.004
AD(MeOH)5	0.959 ± 0.006	1.706 ± 0.025	2.985 ± 0.012	32.409 ± 0.041	58.102 ± 0.011
CP(MeOH)1	0.323 ± 0.002	0.539 ± 0.005	1.508 ± 0.016	2.531 ± 0.004	14.647 ± 0.029
CP(MeOH)2	0.616 ± 0.020	2.406 ± 0.001	3.367 ± 0.001	19.138 ± 0.013	69.782 ± 0.014
CP(MeOH)3	0.878 ± 0.020	3.104 ± 0.001	4.853 ± 0.004	23.307 ± 0.012	73.420 ± 0.001
CP(MeOH)4	0.339 ± 0.012	1.868 ± 0.001	2.094 ± 0.001	2.829± 0.004	22.864 ± 0.005
CP(MeOH)5	5.460 ± 0.008	6.448 ± 0.001	7.488 ± 0.001	9.048 ± 0.006	15.913 ± 0.001
EH(MeOH)1	1.466 ± 0.001	2.029 ± 0.004	4.171 ± 0.001	11.387 ± 0.044	61.330 ± 0.016
EH(MeOH)2	2.368 ± 0.005	5.975 ± 0.047	9.357 ± 0.001	65.84 ± 0.009	80.722 ± 0.016
EH(MeOH)3	0.345 ± 0.013	1.839 ± 0.007	3.218 ± 0.007	27.741 ± 0.031	80.839 ± 0.007
EH(MeOH)4	2.154 ± 0.024	6.122 ± 0.000	8.844 ± 0.001	20.181 ± 0.001	72.184 ± 0.004
EH(MeOH)5	0.314 ± 0.006	0.383 ± 0.002	0.712 ± 0.001	0.932 ± 0.006	4.932 ± 0.052

1=20% aq.MeOH, 2= 40% aq.MeOH, 3= 60% aq.MeOH, 4= 80% aq.MeOH, 5= 100% MeOH.

LEGEND

AD(Ex) = crude extract of *A.digitata* (AD)	**CP(Ex)** = crude extract of *C. procera* (CP)	**EH(Ex)** = crude extract of *E. hirta* (EH)
AD(Hex)m = hexane fraction of AD from 50% aq.MeOH extract	**AD(EtOAc)** = ethyl acetate fraction of AD	**AD(BuOH)** = butanol fraction of AD
CP(Hex)m = hexane fraction of CP from 50% aq.MeOH extract	**CP(EtOAc)** = ethyl acetate fraction of CP	**EH(BuOH)** = butanol fraction of EH
EH(Hex)m = hexane fraction of EH from 50% aq.MeOH extract	**EH(EtOAc)** = ethyl acetate fraction of EH	**EH(BuOH)** = butanol fraction of EH
AD(Hex) = hexane fraction of AD from soxhlet extraction	**CP(Hex)** = hexane fraction of CP from soxhlet extraction	**EH(Hex)** = hexane fraction of EH from soxhlet extraction
AD(DCM) = dichloromethane fraction of AD	**CP(DCM)** = dichloromethane fraction of CP	**EH(DCM)** = dichloromethane fraction of EH
AD(MeOH) = methanol fraction of AD	**CP(MeOH)** = methanol fraction of CP	**EH(MeOH)** = methanol fraction of EH
AD(MeOH)1 = 20% MeOH fraction of AD from SPE	**CP(MeOH)1** = 20% MeOH fraction of CP from SPE	**EH(MeOH)1** = 20% MeOH fraction of EH from SPE
AD(MeOH)2 = 40% MeOH fraction of AD	**CP(MeOH)2** = 40% MeOH fraction of CP	**EH(MeOH)2** = 40% MeOH fraction of EH
AD(MeOH)3 = 60% MeOH fraction of AD	**CP(MeOH)3** = 60% MeOH fraction of CP	**EH(MeOH)**3 = 60% MeOH fraction of EH
AD(MeOH)4 = 80% MeOH fraction of AD	**CP(MeOH)4** = 80% MeOH fraction of CP	**EH(MeOH)4** = 80% MeOH fraction of EH
AD(MeOH)5 = 1000% MeOH fraction of AD	**CP(MeOH)5** = 100% MeOH fraction of CP	**EH(MeOH)5** = 100% MeOH fraction of EH

SPE = solid phase extracts

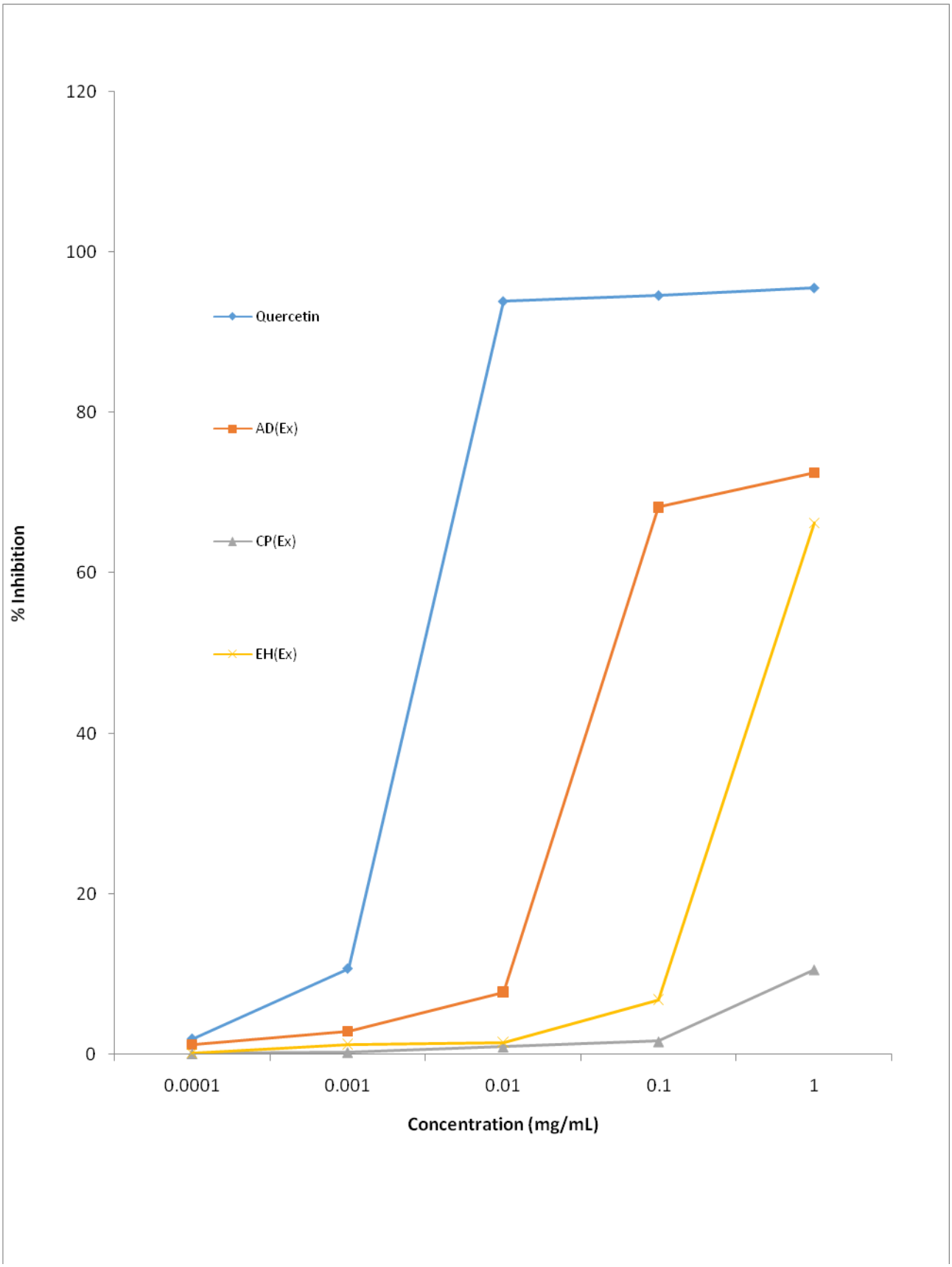


Figure 86: Percentage Inhibition of the DPPH cation radicals by the crude extracts of *A. digitata* (AD), *E.hirta* (EH) and *C. procera* (CP) and quercetin

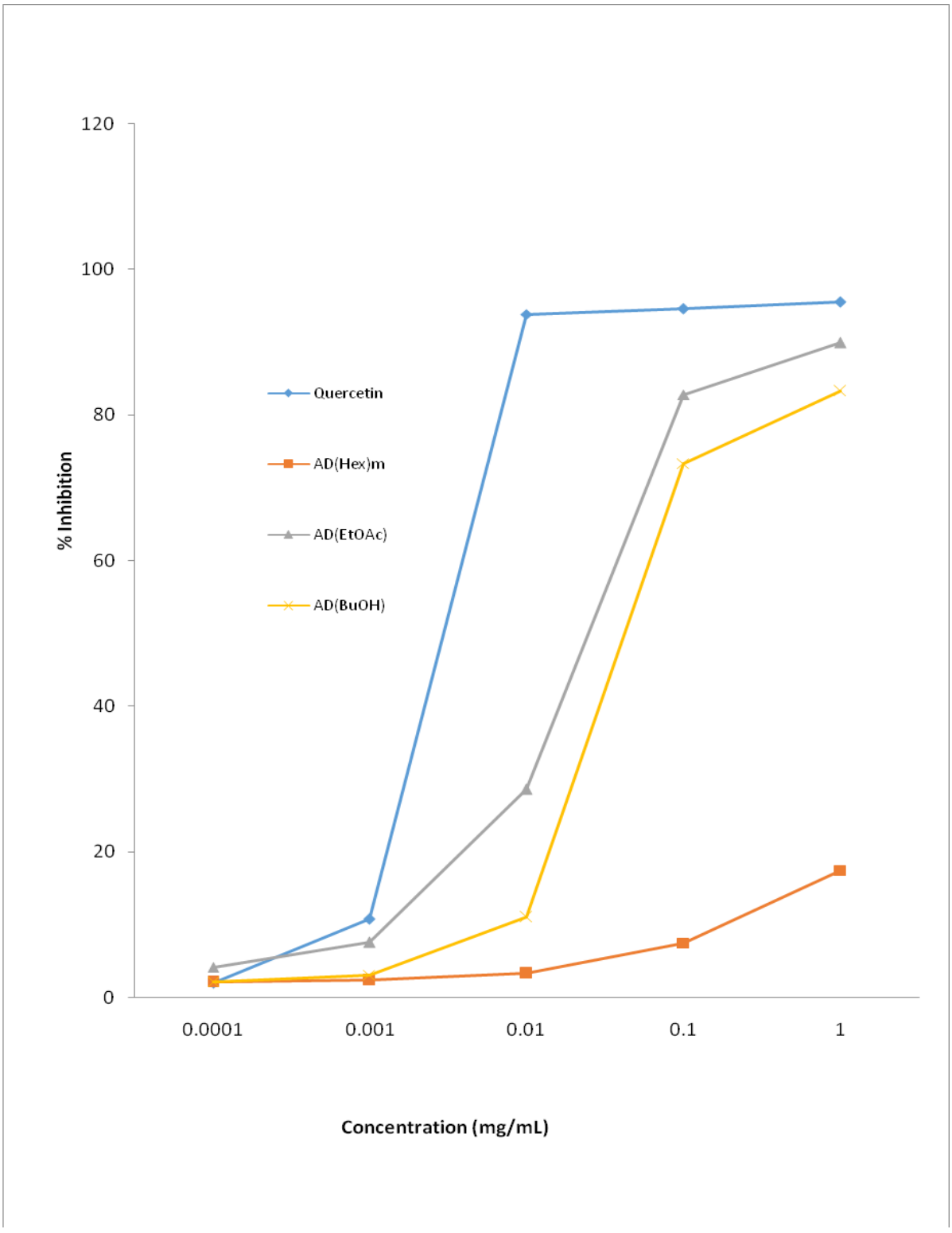


Figure 87: Percentage Inhibition of the DPPH cation radicals by the hexane {(Hex)m} , ethylacetate (EtOAc) and butanol (BuOH) fractions of 50% aq. methanol leaf extracts of AD and quercetin

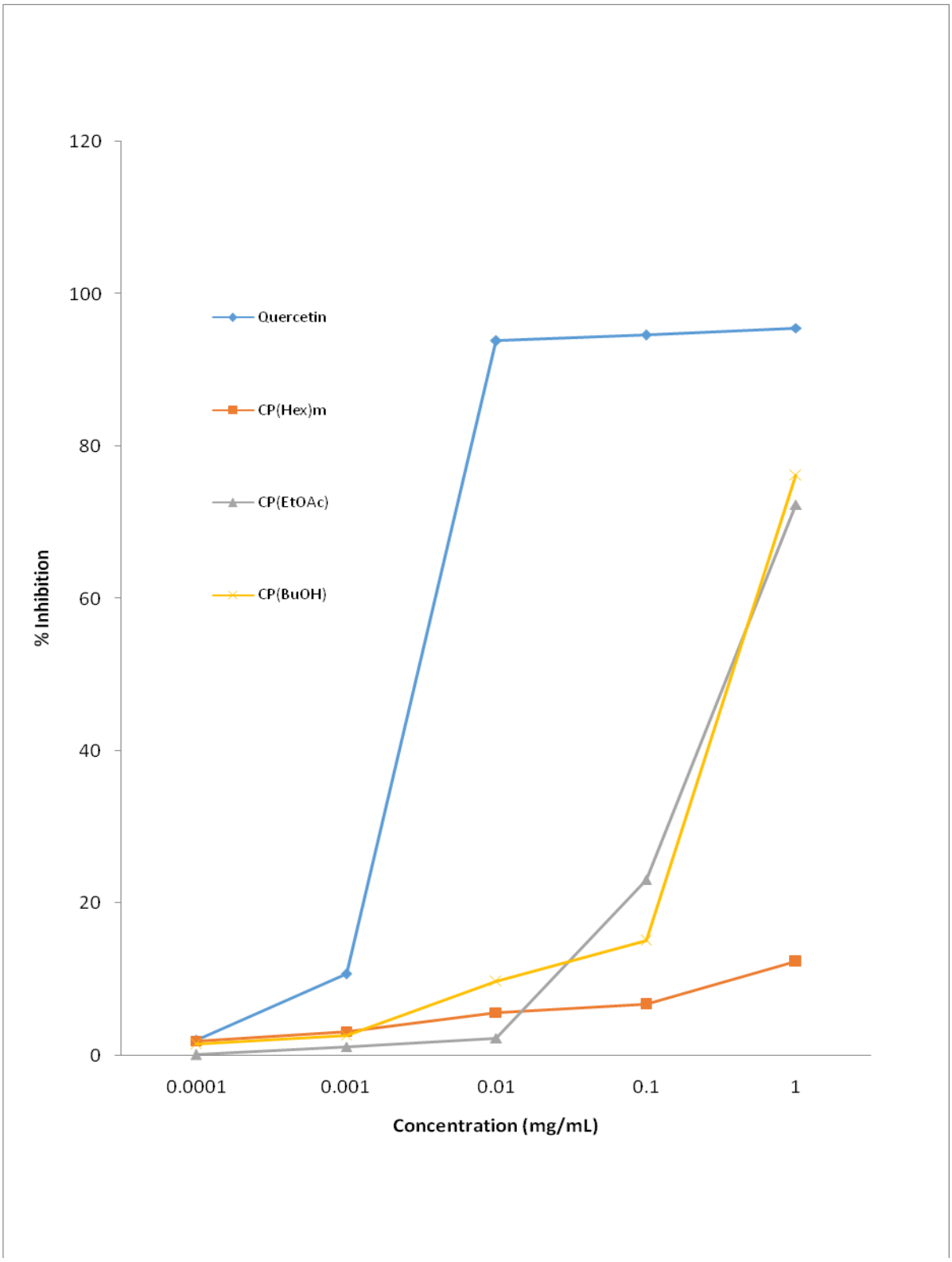


Figure 88: Percentage Inhibition of the DPPH cation radicals by (Hex)m, EtOAc and BuOH fractions of 50% aqueous methanol leaf extract of *C. procera* and quercetin.

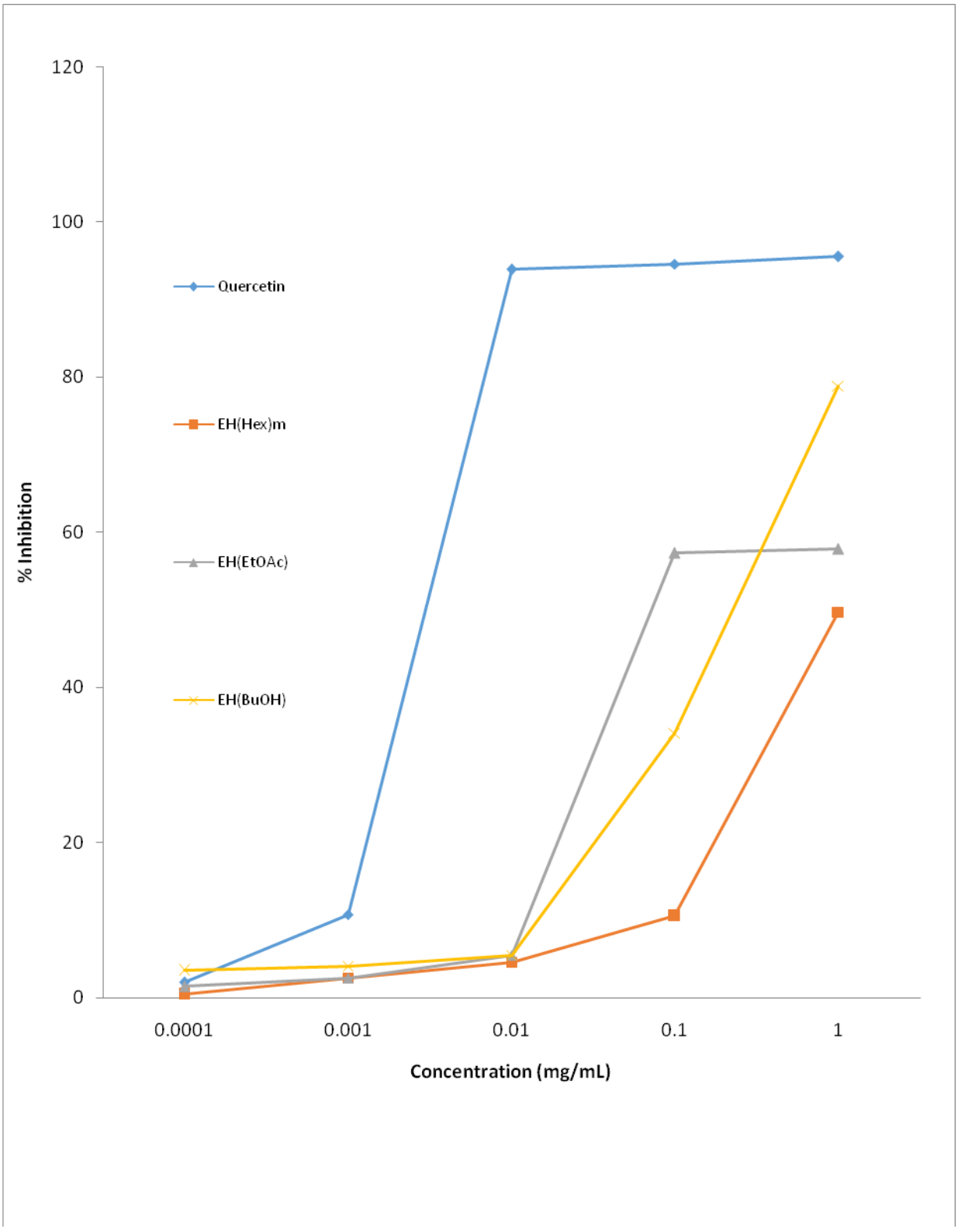


Figure 89: Percentage Inhibition of the DPPH cation radicals by the hexane, ethylacetate and butanol 50% aq.MeOH aerial parts extracts of *E. hirta* and quercetin

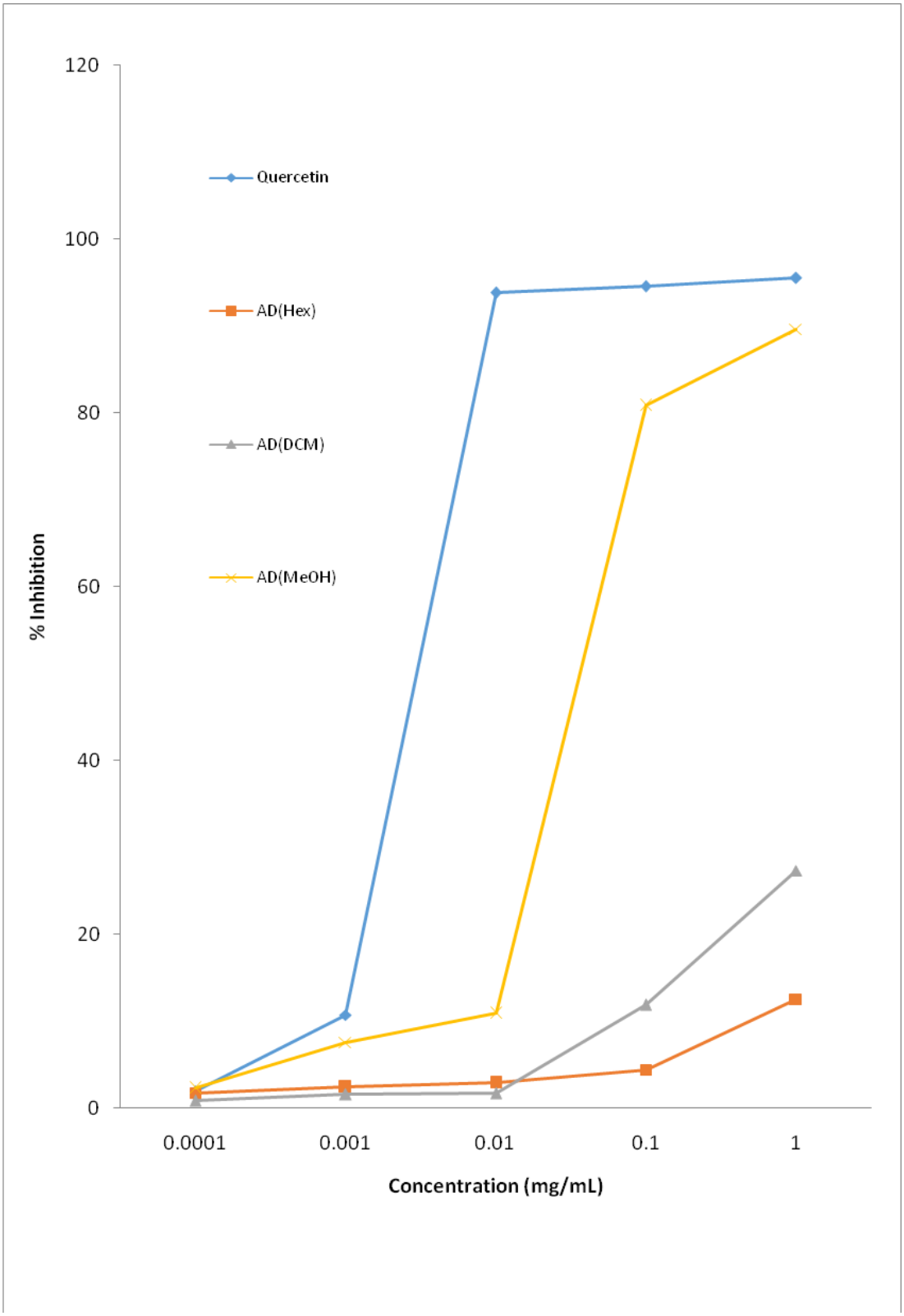


Figure 90: Percentage Inhibition of the DPPH cation radicals by the hexane, dichloromethane (DCM) and methanol soxhlet leaf extracts of *A. digitata* and quercetin

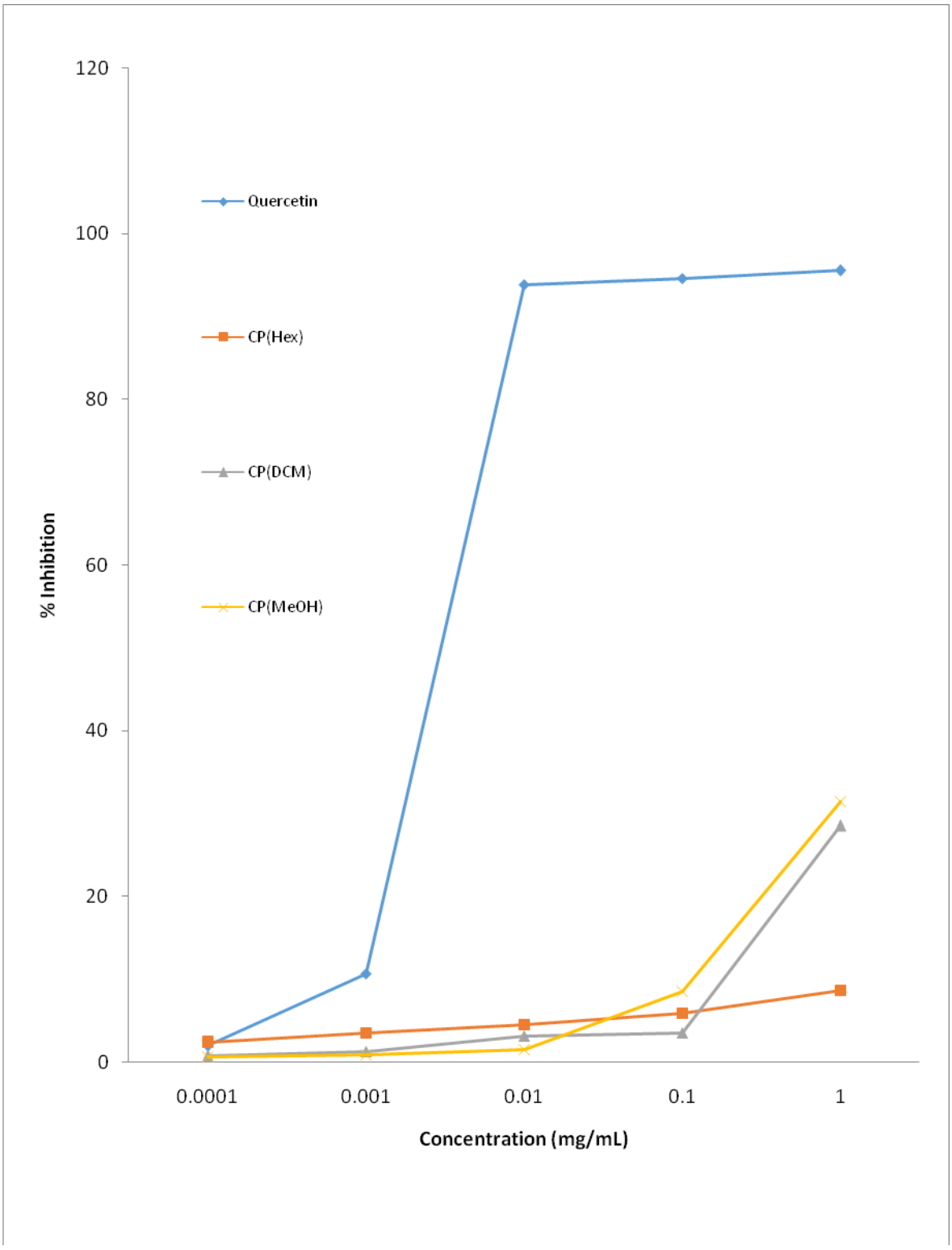


Figure 91: Percentage Inhibition of the DPPH cation radicals by the hexane, DCM and MeOH soxhlet leaf extracts of *C. procera* and quercetin

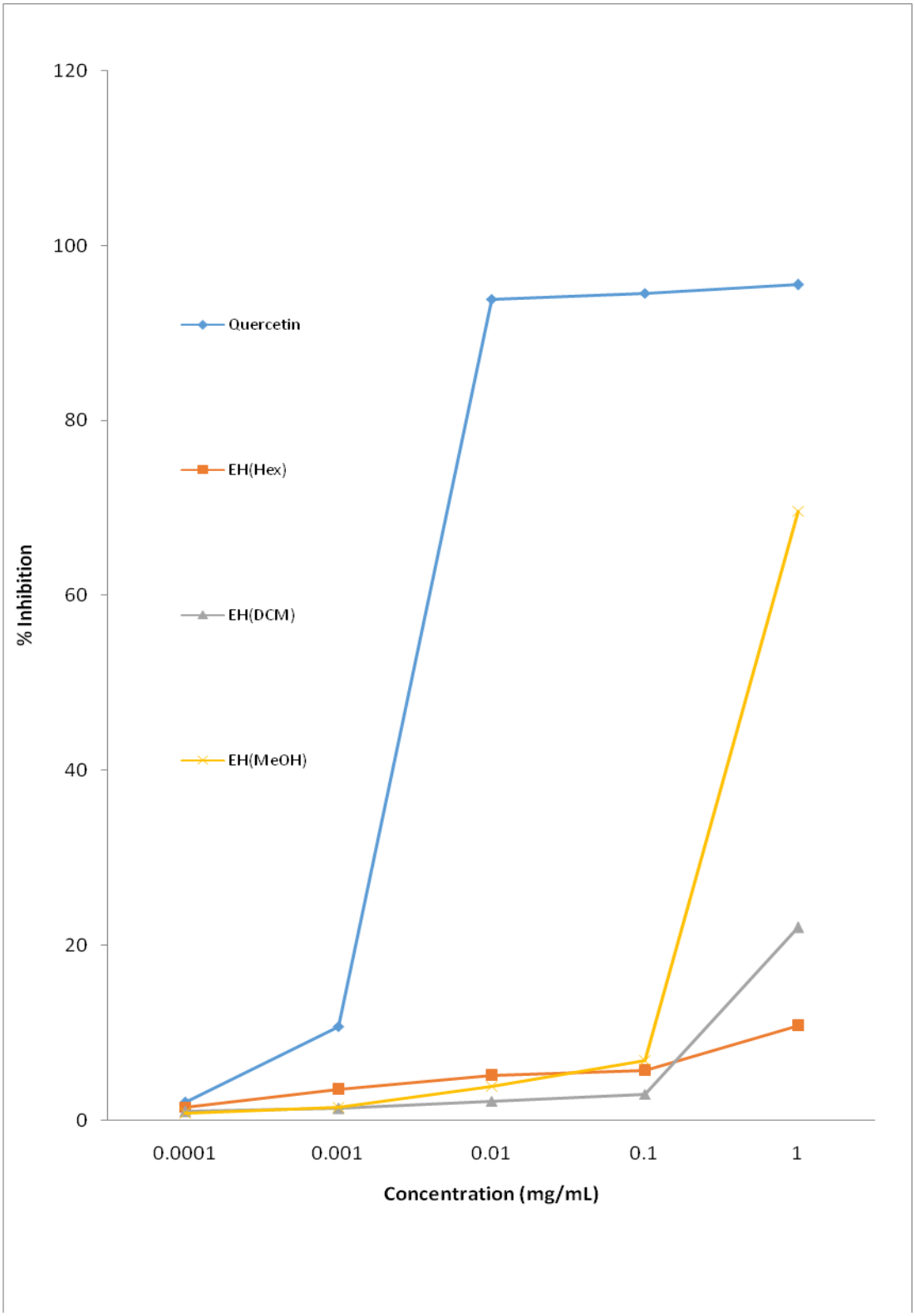


Figure 92: Percentage Inhibition of the DPPH cation radicals by the hexane, dichloromethane (DCM) and methanol soxhlet aerial parts extracts of *E. hirta* and quercetin

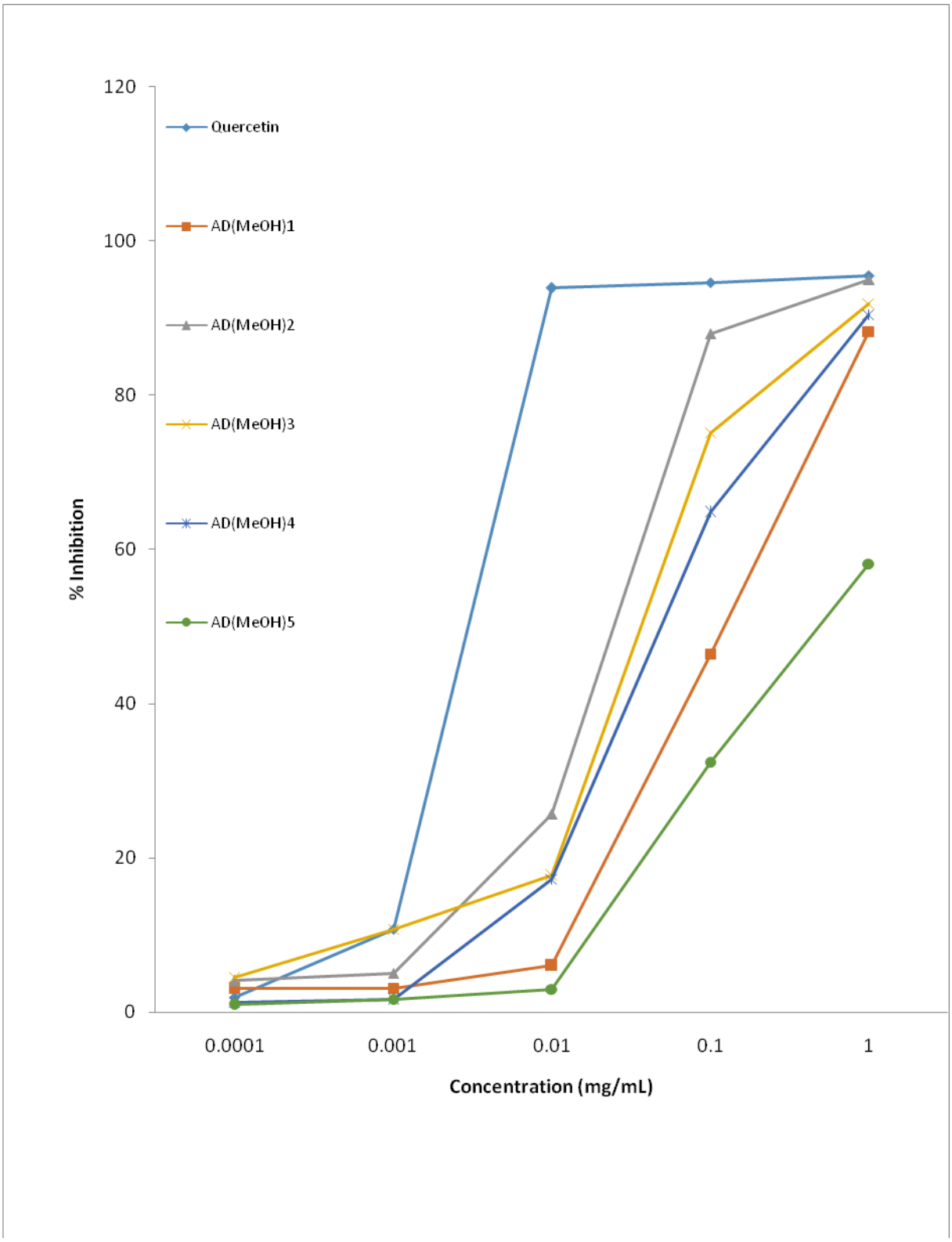


Figure 93: Percentage Inhibition of the DPPH cation radicals by the MeOH soxhlet SPE leaf extracts of *A. digitata* and quercetin

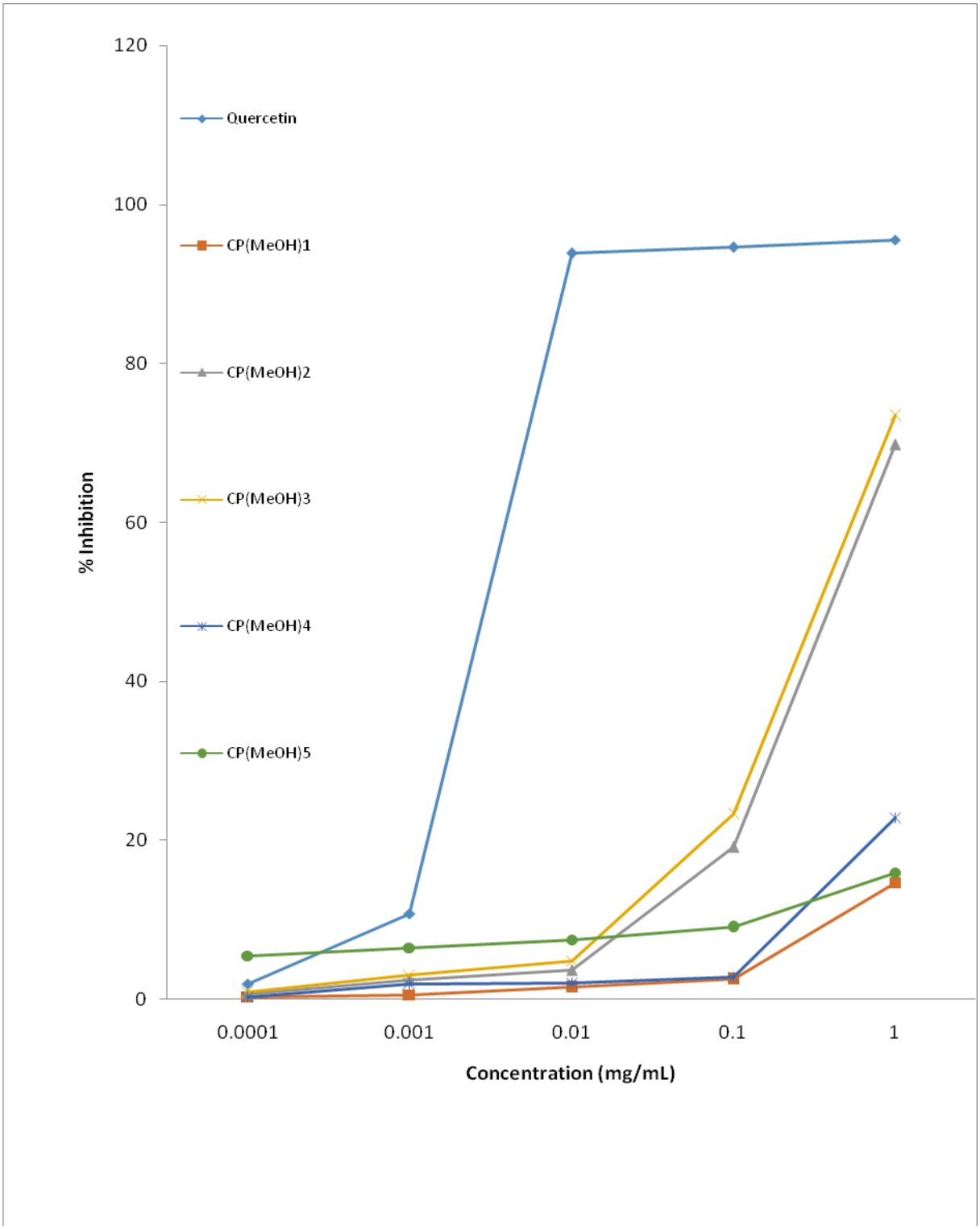


Figure 94: Percentage Inhibition of the DPPH cation radicals by the MeOH soxhlet SPE leaf extracts of *C. procera* and quercetin

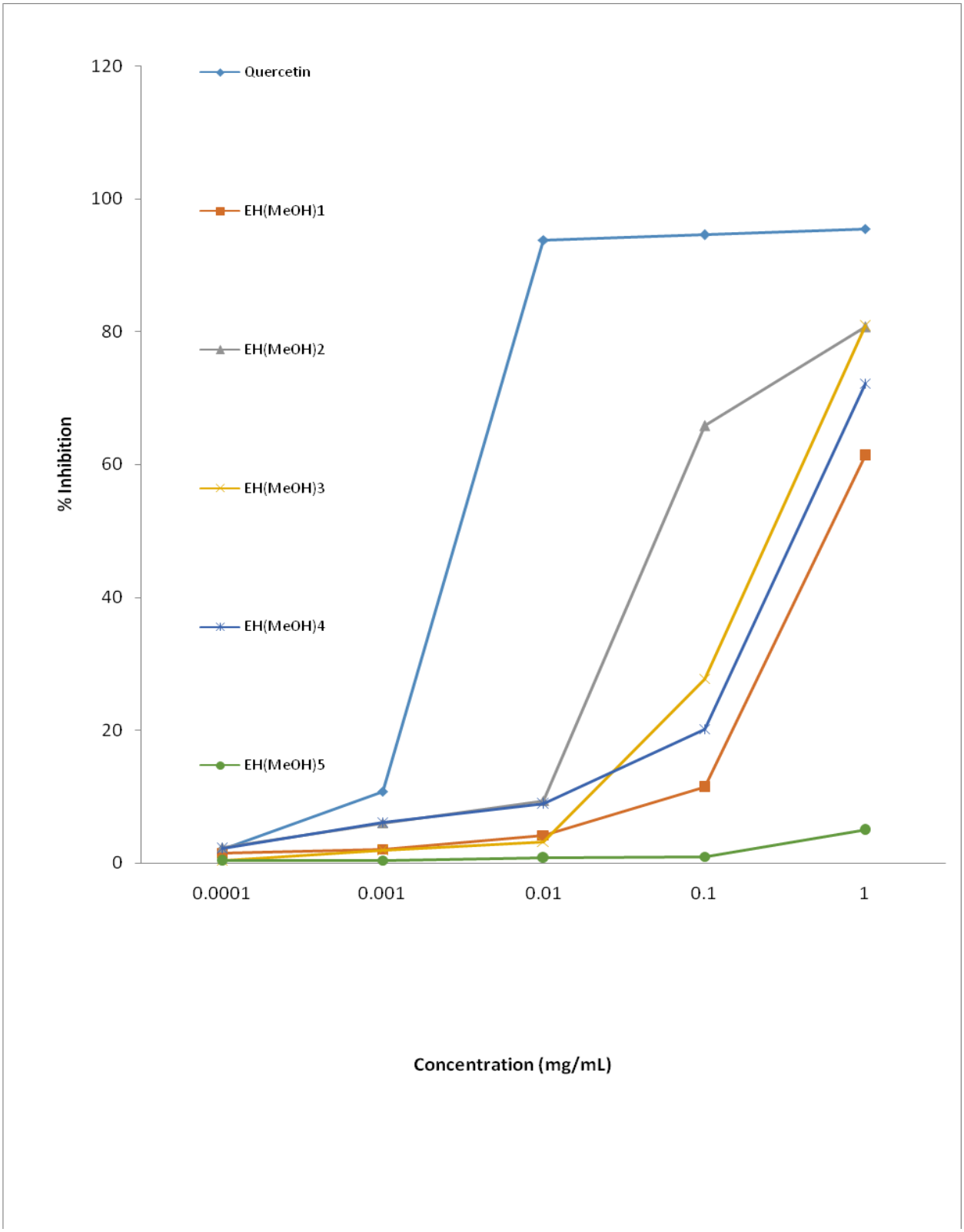


Figure 95: Percentage Inhibition of the DPPH cation radicals by the MeOH soxhlet SPE aerial parts extracts of *E. hirta* and quercetin

Table 24: 50% Inhibition Concentration (IC_{50}) of the extracts and fractions of AD, CP and EH (mg/mL)

50% aq.MeOH crude extracts/IC_{50}	**Fractions from crude 50% aq.MeOH Extracts/ IC_{50}**	**Soxhlet Extracts/ IC_{50}**	**Fractions from Solid Phase Extraction/IC_{50}**
AD(Ex) $36.88x10^{-2}$	$AD(Hex)_m$ NA AD(EtOAc) $3.476x10^{-2}$ AD(BuOH) $6.425x10^{-2}$	AD(Hex) NA AD(DCM) NA AD(MeOH) $4.023x10^{-2}$	AD(MeOH)1 $5.422x10^{-2}$ AD(MeOH)2 $2.933x10^{-2}$ AD(MeOH)3 $2.958x10^{-2}$ AD(MeOH)4 $3.031x10^{-2}$ AD(MeOH)5 $74.64x10^{-2}$
CP(Ex) NA	$CP(Hex)_m$ NA CP(EtOAc) $47.69x10^{-2}$ CP(BuOH) $26.22x10^{-2}$	CP(Hex) NA CP(DCM) NA CP(MeOH) NA	CP(MeOH)1 NA CP(MeOH)2 $52.38x10^{-2}$ CP(MeOH)3 $34.82x10^{-2}$ CP(MeOH)4 NA CP(MeOH)5 NA
EH(Ex) $66.03x10^{-2}$	$EH(Hex)_m$ NA EH(EtOAc) $79.03x10^{-2}$ EH(BuOH) $25.47x10^{-2}$	EH(Hex) NA EH(DCM) NA EH(MeOH) $56.12x10^{-2}$	EH(MeOH)1 $72.13 x10^{-2}$ EH(MeOH)2 $8.560x10^{-2}$ EH(MeOH)3 $6.882x10^{-2}$ EH(MeOH)4 $48.6x10^{-2}$ EH(MeOH)5 NA
∗**Quercetin** $0.52 x10^{-2}$			

NA = Not Applicable as the Inhibition concentration is < 50,
∗ = Quercetin standard
1, 2, 3, 4, 5 = 20, 40, 60, 80% aq. MeOH and 100% MeOH respectively

4.7.2 Anti-inflammatory assay for the methanol extracts of *A. digitata* (AD), *C. procera* (CP) and *E. hirta* (EH)

The anti-inflammatory activities of the methanol extracts of AD, CP and EH were evaluated on the basis of their inhibition of NO (measured as nitrite which is the stable oxidation product) in LPS - activated macrophage cells. Low percentage nitrite production indicates high activity. The percentage nitrite production and the percentage anti-inflammatory activities of the extracts for AD, CP and EH at 25, 50 and 100 μg/mL concentrations are represented with bar charts presented in Figures 83 - 85. Generally, the nitrite production was in the range of 21.373– 86.621% hence 13.379 – 78.627% anti-inflammatory activity. *A. digitata* gave a percentage nitrite production of 21.373 – 69.674%. The range for CP was 56.600 – 86.621% while EH was in the range of 28.338 – 68.807%. Lowest percentage nitrite production (21.373%) indicating highest anti-inflammatory activity (78.627%) was observed at 100 μg/mL of *A.digitata* (Figure 96). Lipopolysaccharide (LPS) without the extracts was used as negative control and it recorded 100% nitrite production hence 0% anti-inflammatory activity as shown in Figures 96 - 98.

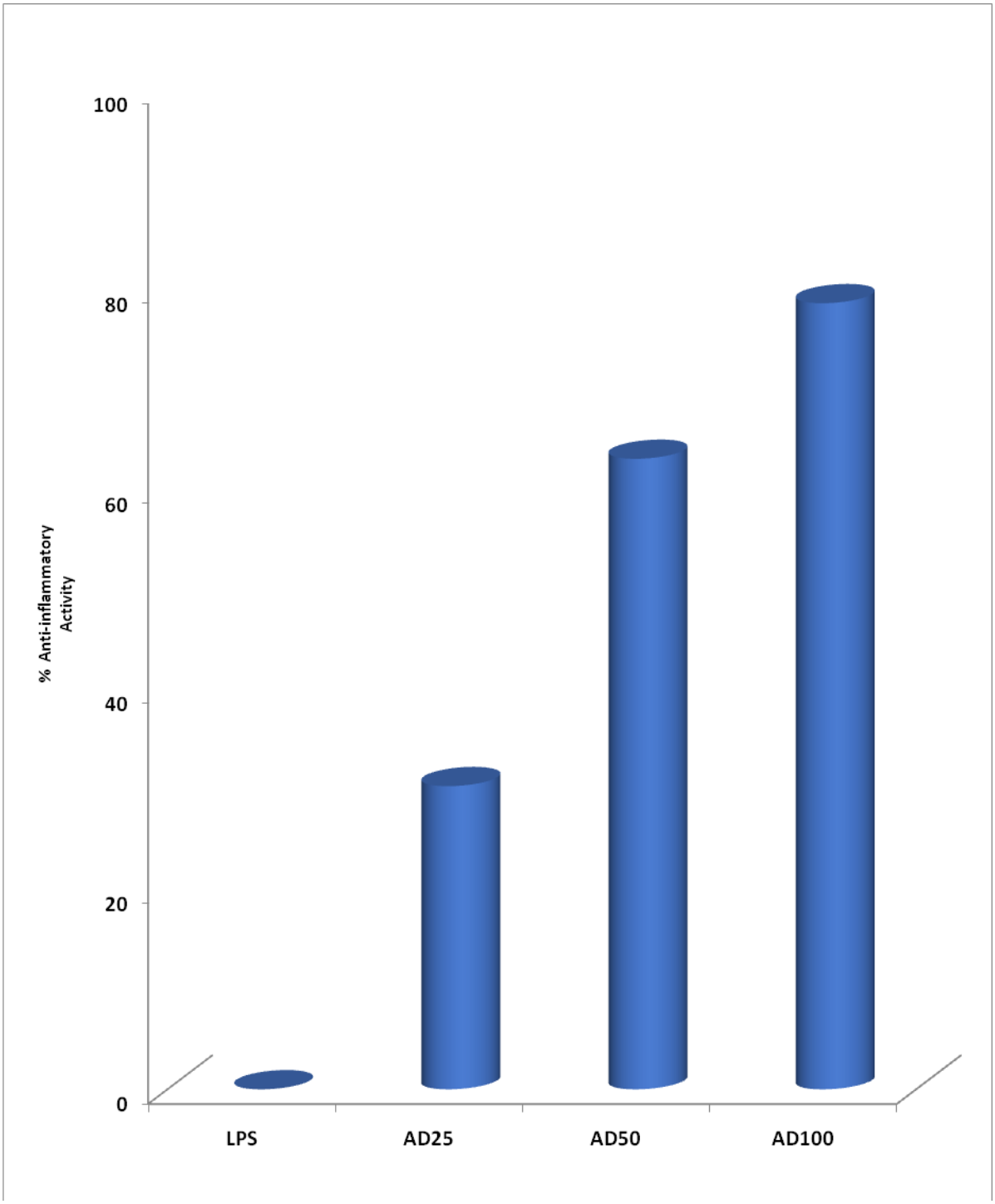


Figure 96: Chart of percentage anti–inflammatory activities of *A. digitata* at different concentrations in comparison with LPS (positive control)

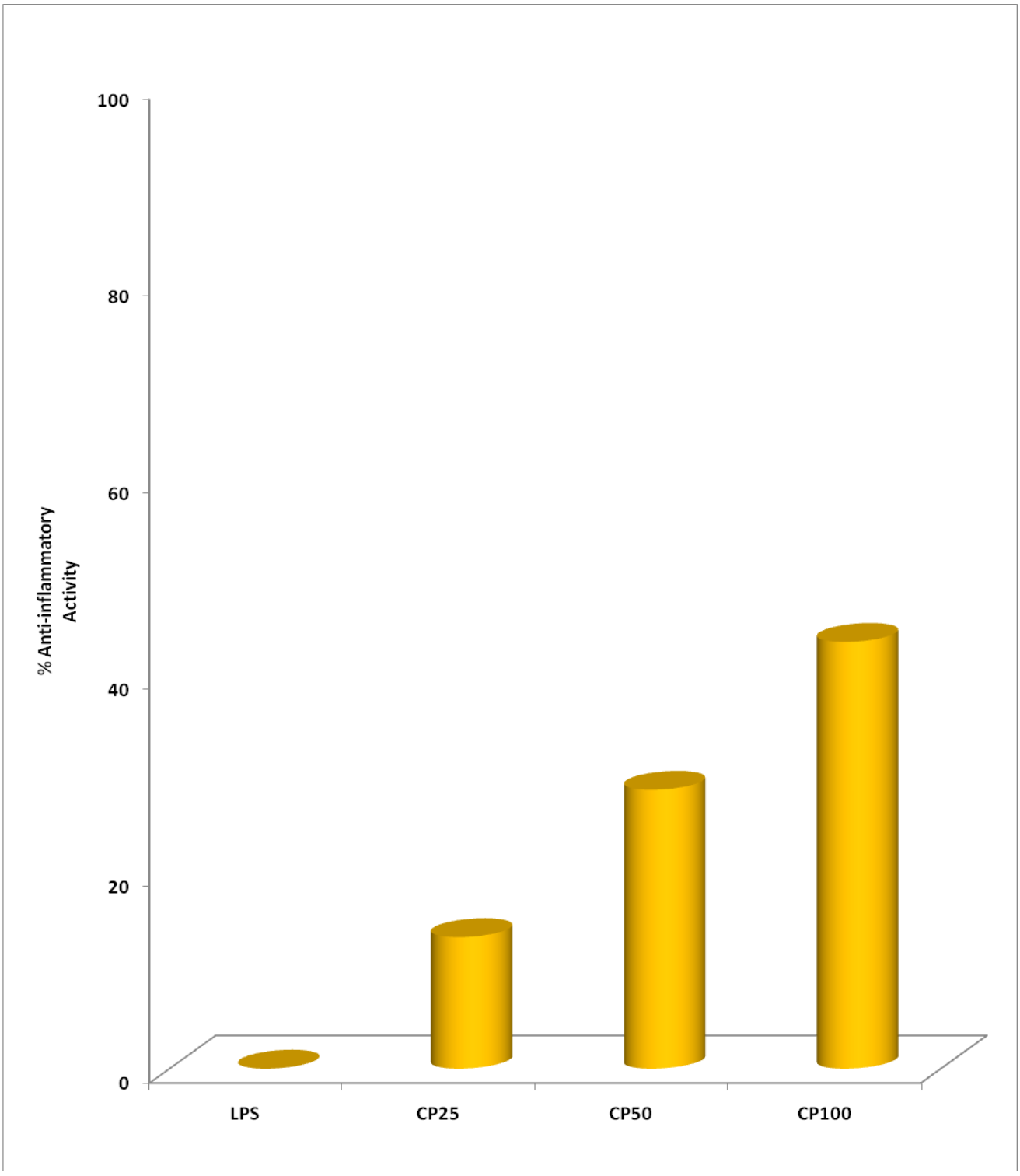


Figure 97: Chart of percentage anti-inflammatory activities of *C. procera* at different concentrations in comparison with LPS (positive control)

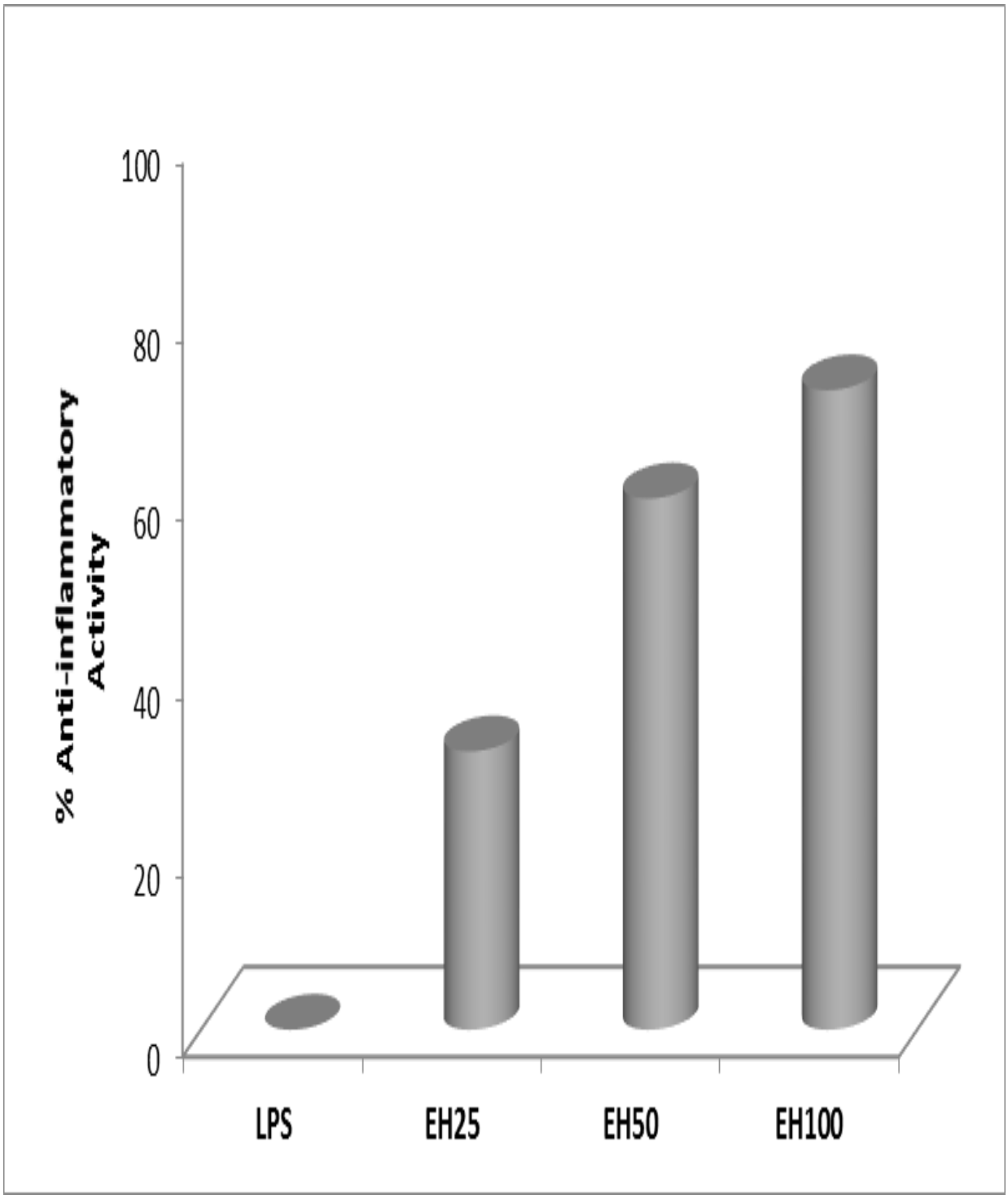


Figure 98: Chart of percentage anti-inflammatory activities of *E. hirta* at different concentrations in comparison with LPS (positive control)

4.7.3 *In vitro* antibacterial activities for soxhlet extracts of *A. digitata*, *C. procera* and *E. hirta*

The results of the *in vitro* antibacterial activities of hexane (Hex), dichloromethane (DCM) and methanol (MeOH) fractions of *A. digitata (*leaves), *C. procera* (leaves) and *E. hirta* (aerial parts) obtained after soxhlet extractions are presented in Table 26. The Minimum Inhibition Concentration (MIC) values for the extracts obtained for the bacteria species represent their antibacterial activities and lower MIC implies higher activity.

Hexane, dichloromethane and methanol extracts of *A.digitata* showed no activity against *A. baumannii*, *E.coli* and *P.aeruginosa*. However, all the extracts of AD showed appreciable activities against *B.cereus* (MIC 1.25 – 2.50 mg/mL) and *S.aureus* (1.25 – 5.00 mg/mL). AD(MeOH) (1.25 mg/mL) exhibited the highest activity for both *B. cereus* and *S. aureus.* However, it exhibited lower activity against *B. cereus* than Ciprofloxacin (MIC 0.63 mg/mL).

No activity was exhibited by the hexane, DCM and MeOH extracts of *C. procera* against *E. coli* and *P.aeruginosa* but the three extracts exhibited appreciable activities against *B.cereus* (MIC 0.63 – 5.00 mg/mL). CP(MeOH) had the highest activity against *B. cereus* among all the extracts. Only CP(Hex) (5.0 mg/mL) showed activity against *A. baumannii* among the different extracts of the three plants. CP(Hex) was the only extract of *C. procera* (5.00 mg/mL) that showed activity against *S. aureus* (Table 26).

The Hex, DCM and MeOH extracts of *E.hirta* showed no activity against *A. baumannii*, *B. cereus* and *E.coli*. However, the three extracts of *E. hirta* demonstrated appreciable activities for *S. aureus* (MIC 1.25 – 5.00 mg/mL). The highest activity was exhibited by EH(MeOH) (MIC 1.25 mg/mL). Only EH(Hex) (MIC 2.5 mg/mL) exhibited activity against *P. aeruginosa*. AD(MeOH) and EH(MeOH) had the highest activity against *S. aureus* among all the extracts and ciprofloxacin as shown in Table 26.

Table 25: Minimum Inhibition Concentration (MIC) of plant extracts against the pathogenic bacteria by Resazurin microtitre dilution assay

PLANT NAMES	EXTRACTS	MIC (mg/mL)				
		A b	B c	E c	P a	S a
Adansonia digitata (AD)	AD(HEX)	-	2.50	-	-	2.50
	AD(DCM)	-	2.50	-	-	5.00
	AD(MeOH)	-	1.25	-	-	1.25
Calotropis procera (CP)	CP(HEX)	5.00	2.50	-	-	5.00
	CP(DCM)	-	5.00	-	-	-
	CP(MeOH)	-	0.63	-	-	-
Euphorbia hirta (EH)	EH(HEX)	-	-	-	2.50	5.00
	EH(DCM)	-	-	-	-	5.00
	EH(MeOH)	-	-	-	-	1.25
Ciprofloxacin		1.25	0.63	0.37	2.50	2.50

A b = *Acinetobacter baumannii;* B c = *Bacillus cereus);* E c = *Escherichia coli);*
P a = Pseudomonas aeruginosa;* S a= *Staphylococcus aureus
HEX = Hexane; DCM = Dichloromethane; MeOH = Methanol
(-) = no activity above 5 mg/mL

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

Samples obtained from the plant materials include the following:

i. Essential oil samples.

ii. The 50% methanol/water extracts which were sequentially extracted with hexane, ethyl acetate (EtOAc) and butanol (BuOH) to give non-polar, medium polar and polar fractions.

iii. Soxhlet extracts using methanol (MeOH), dichloromethane (DCM) and hexane as solvents.

iv. Solid phase extracts (SPE) from the methanolic soxhlet extracts in (iii) which were fractionated using 20, 40, 60, 80% aq. MeOH and 100% methanol.

Essential oil samples were obtained from the leaves of *Adansonia digitata* (AD), *Calotropis procera* (CP), *Coix lacryma-jobi* (CL), *Datura metel* (DM), *Deinbollia pinnata* (DP), aerial parts of *Euphorbia hirta* (EH) and the roots of *Pterocarpus osun* (PO). The constituent compounds in these samples which have been found to exhibit anti-inflammatory activities include:

a) Phytol, an isomer of 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol which is an anti-inflammatory **(**Silva *et al.,* 2014; Hulqvist *et al.,* 2006) and antitumor (Kim *et al.,* 2015) compound was present in the leaves of AD, CP, CL, DM, DP and aerial parts of EH.

b) Poly Unsaturated Fatty Acids (PUFA) such as 9, 12, 15-octadecatrienoic acid (Z, Z, Z) and 9, 12-octadecadienoic acid (Z, Z) - present in the essential oil samples of AD, CL and DM are good antioxidants **(**Yessoufou *et al.,* 2015) and anti-inflammatory compounds (Zhao *et al.,* 2005) thus may be useful in the management of asthma by inhibiting pro-inflammatory events (Henricks and Nijkamp, 2001).

c) Saturated fatty acids (SFA) which include dodecanoic-, tetradecanoic-, pentadecanoic-, n-hexadecanoic-, heptadecanoic- and octadecanoic acids were found to be present in all the plants investigated except CP. n-Hexadecanoic acid is anti-inflammatory (Aperna *et al*, 2012) and is known to have antibacterial and antifungal activities (McGaw *et al.*, 2002; Seidel &Tailor (2004) and thus may also give relief to asthmatic patients when bacteria are the causative agents.

d) Oleic acid which is an unsaturated fatty acid has anti-inflammatory, antibacterial, antifungal, antioxidant and antitumor activities (Cho *et al.*, 2010; Carrillo *et al*., 2012). It was found present in EH and may be useful in the management of asthma.

e) Squalene, which is an isoprenoid has anti-inflammatory, antioxidant and antitumor activities and was found to be present in AD and may be relevant in the management of asthma.

Thus, these known bioactivities in the constituent compounds of the essential oils from the seven plants lend support to their use in the management of asthma in Alternative Medicine (Odugbemi, 2007).

Adansonia digitata, Calotropis procera and *Euphorbia hirta* were chosen for further studies because they had the highest values of known anti-inflammatory, antioxidant and antibacterial compounds as shown in Table 17.

Warra and Sheshi, 2015 analysed *Adansonia digitata* (AD) from Kebbi State. Both analyses were by GC-MS but there were differences in the plant parts and methods of extraction used. The hydrodistillation method as used in this investigation is a standard method for the extraction of essential oils (Zuzarte and Salgueiro, 2015) while Warra and Sheshi used the soxhlet method.

The essential oil analyses of *C. procera* as contained in this report was on the same plant parts (leaves) as in those reported by Kubmarawa & Ogunwande, 2008 and Monrokola *et al*.,

2011. The conventional hydrodistillation method whereby the essential oils are collected once after the extraction process was adopted in the literature reports. The method used in this report involves the collection of the essential oils in fractionated hourly modes in addition to the normal collection after 4 hours. The hourly mode of collection has been useful in detecting the presence of minor components such as tetradecanal, isophytol and docosanol in the essential oil. Phytol, which was found present in high yield in this report was also reported by Kubmarawa and Ogunwande, 2008, though the yield was not stated.

The GC-MS method of analyses which was adopted in this report for aerial parts essential oil of *E. hirta* was also reported in literature. However, Chitra *et al*., 2011 screened the crude methanolic leaf extracts and Suresh *et al.,* 2012 reported the analyses of crude ethanolic extracts (not essential oils) hence the results cannot be compared.

The UV-Vis spectra of the scanned chromatographic peaks obtained from the HPLC analyses of SPE fractions from the leaves of *Adansonia digitata, Calotropis procera* and aerial parts of *Euphorbia hirta* showing different wavelengths of maximum absorption revealed the presence of some functional groups hence groups of compounds in the plants. However, the positions of the absorbance maxima of the chromophores are not fixed but dependent upon some factors which include their molecular environments. Flavonoids (250 – 370 nm) were found present in *A. digitata*, *C. procera* and *E. hirta;* fatty acids, RCOOH, (200 nm), such as n- hexadecanoic acids were identified in *A. digitata* and *E. hirta* but were absent in *C. procera*; aldehydes, RHC=O (293 nm), which include hexadecanal were found to be present in *C. procera* and *E. hirta;* ketones, RR'C=O (271 – 273 nm) for example, 6,10, 14-trimethyl, 2-Pentadecanone were present in *A. digitata*, *C. procera* and *E. hirta;* alcohols, ROH (200 – 250 nm) with an example, phytol, were also identified in the three plants investigated. The presence of the functional groups agrees with the classes of some of the compounds identified

from the GC-MS analyses of the essential oil samples from the leaves of *Adansonia digitata*, *Calotropis procera* and aerial parts of *Euphorbia hirta*.

The scanned peak #17 from the chromatogram of the ethyl acetate fraction of *A. digitata* (Figure 80) gave a UV-Vis spectrum with maximum absorptions at 200.8, 258.4 and 374.7 nm (Figure 81) which were the same as those for standard reference UV-Vis spectrum for quercetin and comparable to the absorption bands of 257 and 373 nm which refer to the conjugations in B and A ring of quercetin as reported by Timbola *et al.,* 2006.

The isolated crystalline yellow compound gave a melting point of 314.5^{o}C (lit.316^{o}C) and retention time of 19.367 mins (Figure 82) which was also comparable to that of the standard quercetin that eluted at 19.522 mins under the same conditions (Figure 83).

The mass spectral data gave the molecular peak at m/z 301.0351 which was comparable to that of the standard quercetin with molecular peak at m/z 301.0354 (Figure 84 and 85) hence the characteristics of the isolated compound were established to be for quercetin from the melting point, retention time, UV-Vis spectrum and mass spectral data.

The antioxidant (free radical scavenging) activities of the extracts and fractions of *A. digitata*, *C. procera* and *E. hirta* were evaluated based on their abilities to scavenge the synthetic 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

Generally, the antioxidant activities of the extracts and fractions of the plants studied were found to be concentration - dependent as shown in Table 23. This trend agrees with the report of Zeidan and Oran (2014). The results in the Table also show that quercetin solution of concentrations in the range 1 x 10^{-4} – 1 mg/mL had stronger antioxidant activity than the tested extracts. This was to be expected because the extracts were not pure but mixtures of different compounds. However, the degrees of purity of extracts were reflected (Tables 23 and 24; Figure 86 -95) as the more the plants were purified (fractionated), the higher the

antioxidant activities as depicted in the trend: fractions from SPE > fractions from 50% aq. MeOH > soxhlet extracts > crude extracts for AD and EH; fractions from 50% aq. MeOH > fractions from SPE > soxhlet extracts > crude extracts for CP. *A. digitata* exhibited the highest antioxidant activity while *C. procera* had the least hence AD > EH > CP. The 40% and 60% aq. MeOH fractions of AD and EH respectively and the butanol fraction of CP with lowest IC_{50} (Table 24) hence highest antioxidant activities were the most potent when compared with the other extracts and fractions for each of the plants. That might be attributed to the complex nature of the plant constituents. The results of this assay also showed that the antioxidant activities of the extracts were mainly affected by the polarity of the solvents used for the extraction more than the extraction methods and these were in agreement with literature (Aedil *et al.,* 2012).

The MeOH and BuOH (polar) extracts and fractions exhibited the highest antioxidant activities in most cases. For example, EH(Hex) and EH(DCM) had no IC_{50} whereas EH(MeOH) had an IC_{50} of 56.12×10^{-2} mg/mL (Table 24). However, AD(EtOAc) exhibited the highest antioxidant activity among the 50% aq. MeOH extracts/fractions and the extracts from soxhlet extraction.

The fractions obtained from the extracts showed higher antioxidant activities than the crude extracts. The crude extract of AD gave an IC_{50} of 36.88×10^{-2} mg/mL but its ethyl acetate fraction gave a higher antioxidant activity with IC_{50} of 3.476×10^{-2} mg/mL (Table 24) hence two active fractions can be combined to further increase the activities. Generally, all the plant extracts and their fractions investigated had varying degrees of antioxidant activities which can reduce the oxidative stress that accompanies asthma and thus can reduce the symptoms of asthma and improve pulmonary functions (Al-Jawad *et al.*, 2010).

The antioxidant assay of *A. digitata* in this report was carried out on the leaf crude extracts, fractions from the crude extracts, soxhlet extracts and SPE fractions from the soxhlet

methanol extracts. The evaluation of the antioxidant activities by Ayele *et al.,* 2013 was on crude methanolic leaf extract of *A.digitata* from Ethiopia. Comparing the values of IC_{50} which is an indication of antioxidant activity, the plant from Ethiopia was more active than that from Nigeria. The difference in the results may be due to the climatic and ecological differences between the two countries.

Generally, DPPH assay was adopted in this report and in many literature works such as those reported by Yesmin *et al.,* 2008, Joshi *et al.,* 2009 and Mohanraj & Usmani, 2012 on the antioxidant activities of *C. procera* from Bangladesh and India respectively. The test is simple and rapid and needs only a UV-Vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening (Prior *et al*., 2005). The antioxidant activity of methanolic leaf extract of *C. procera* from Bangladesh as reported by Yesmin *et al*., 2008 was higher than those from India. The Nigerian *C. procera* had low activity as its percentage inhibition was lower than 50% hence it was recorded as NA (no activity). This could also be attributed to the different climatic and environmental conditions that exist at different sources of the plants as Kaur *et al.,* 2016 recently demonstrated that changing environmental conditions significantly affect the bioactive constituents, which accumulate as a defence strategy by plants.

The DPPH method which was used in this work for the antioxidant assay of extracts/fractions from Soxhlet, SPE and 50% aq.MeOH of aerial parts of *E. hirta* was also contained in some literature reports. Sharma *et al*., 2014 evaluated the antioxidant potency of ethanolic whole plant extracts of the plant from India. Asha *et al.,* 2016 evaluated the antioxidant activity of aqueous, methanolic and ethanolic leaf extracts of *E. hirta* also from India. Basma *et al.,* 2011, screened the crude methanolic leaf, flower, stem and root extracts of *E. hirta* from Malaysia for antioxidant activities while that of crude aqueous aerial parts extracts of the

plant from Abidjan was reported by Fofie *et al*., 2013. These results cannot be compared because of the differences in the sources of the plants and extracts used.

The results of anti-inflammatory assay showed that anti-inflammatory activities increased in a dose-dependent manner with increase in concentrations (25, 50 and 100 μg/mL) of the extracts (Figures 96 – 98). This was in agreement with literature (Samad *et al.,* 2014). The highest anti- inflammatory activity was at 100 μg/mL concentration for all extracts of AD, CP and EH. The anti-inflammatory activities of AD and EH appeared very close. For example, at 25 μg/mL, AD and EH had the percentage anti-inflammatory activities of 30.326 and 31.193% respectively and similarly at 50 and 100 μg/mL. However, CP exhibited the lowest anti-inflammatory activity at all the concentrations (Table 25). The extract of *A. digitata* at 100 μg/mL exhibited the highest anti-inflammatory activity (78.627%) while 25 μg/mL of *C. procera* gave the lowest anti-inflammatory activity (13.379%) of the extracts at all the concentrations tested hence the trend: AD > EH > CP. This trend agrees with the composition of anti-inflammatory compounds which include phytol, hexadecanoic acid, polyunsaturated fatty acids, oleic acid and squalene whicn are relevant in the management of asthma and were obtained from the essential oil samples of *A. digitata* leaves (76.97%), *C. procera* leaves (67.72%) and *E. hirta* aerial parts (70.98%) as presented in Table 17.

The method used for the anti-inflammatory activities of *A.digitata* in this study was nitric oxide assay. Ayele *et al*., 2013, carried out the experiment using the same method but on the methanolic leaf extracts of the plant from Ethiopia. From the results, the methanolic leaf extracts of the Nigerian plant as contained in this report had high activity at a lower concentration of 25 μg/mL. The sample from Ethiopia as reported by Ayele *et al*., 2013 was within concentration range of 50-100 mg/mL and had high potency though the actual values

were not stated. The difference in the level of potency may be attributed to geographical, physiological, environmental and genetic factors (Figueiredo *et al*., 2008).

The *in vitro* anti-inflammatory activity of *C. procera* soxhlet methanolic, dichloromethane and hexane leaf extracts evaluated on the basis of their inhibition of NO (measured as nitrite which is the stable oxidation product) in LPS–induced RAW 264.7 cells reported in this study have not been reported in literature to the best of my knowledge.

The anti-inflammatory assay adopted by Arya & Kumar 2005 and Babu & Karki, 2011 involved *in vivo* assay using induced paw oedema in rats.

The *in vitro* determination of the anti-inflammatory activity of *E. hirta* from Taiwan by Shih *et al.,* 2010 involved the same nitric oxide assay as was adopted in this report. There is also similarity in the extracts used. However, there was no comparable results on the *E. hirta* from Taiwan with those from Nigeria as obtained in this report.

Antibacterial results (Table 25) revealed that seven extracts had activities against *S. aureus* while six of them had activities against *B. subtilis. S. aureus* had been implicated in bacterial infections present in asthmatic patients (Tomassen *et al.,* 2013). AD, CP and EH which exhibited antibacterial activities against *S. aureus* are thus potentially useful in the management of asthma. Generally, lowest MIC values hence highest antibacterial activities were observed among the MeOH extracts with the highest being CP(MeOH) which had the MIC of 0.63 mg/mL**.**

The antibacterial screening reported by Kubmarawa *et al.,* 2007 used the agar diffusion method alone to determine the activities of the ethanolic extracts of the aerial parts of *A. digitata* against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.* Additional serial dilution of the extracts were adopted to obtain the minimum inhibition concentrations (MIC) though *A. digitata* recorded no MIC against all the bacterial strains screened. In this report, the leaf methanolic, dichloromethane

(DCM) and hexane extracts of *A. digitata* which were screened using colorimetric resazurin microtitre dilution assay (RMDA) showed activities against some similar bacteria such as *B. cereus* and *S. aureus* with reasonable MICs.

In another report by Masola *et al.,* 2009, the antibacterial activities of ethanolic and aqueous root and stem bark of *A. digitata* were evaluated. However, the results cannot be compared because of the differences in the plant parts used.

Generally, the colorimetric assays gave MIC values which were not reported when agar diffusion method was employed alone. The advantages of the colorimetric microdilution procedure include the generation of MICs, the reproducibility and convenience of having prepared panels, the economy of reagents and space that occurs due to the miniaturization of the test. There is also an assistance in generating computerized reports if an automated panel reader is used (Reller *et al*., 2009).

The colorimetric RMDA which was adopted for the antibacterial screening in this report was not obtained in literature neither for *C. procera* nor *E. hirta* to the best of my knowledge.

There were no similar reports on the other plants *(Datura metel, Coix lacrymajobi, Deinbollia pinnata* and *Pterocarpus osun*).

CHAPTER SIX

6.0 SUMMARY OF FINDINGS AND CONCLUSION

6.1 Summary of Findings

Specific Objectives	Summary of Findings
1. Modification of the collection method in hydrodistillation and GC-MS analyses of the essential oil samples obtained from the listed plants out of which the plants with high percentage of known anti-inflammatory compounds will be selected for detailed study.	Seven plants were selected and essential oil samples were extracted from them in **fractionated modes**. The plants were *Adansonia digitata* (leaves), *Calotropis procera* (leaves), *Coix lacryma-jobi* (leaves), *Datura metel* (leaves), *Deinbolia pinnata* (leaves), *Euphorbia hirta* (aerial parts) and *Pterocarpus osun* (root). *A. digitata*, *C. procera* and *E. hirta* were found to contain among other compounds, appreciable quantities of phytol, n-hexadecanoic acids, polyunsaturated fatty acids, oleic acid and squalene which were compounds that have been reported to exhibit anti-inflammatory activities and were thus selected for further investigations.
2. Fractionation and characterization of the 50% aqueous MeOH and hexane, dichloromethane and MeOH Soxhlet extracts of the selected plants.	The 50% aq. MeOH extraction was followed by sequential extraction (with hexane, EtOAc and butanol). Soxhlet extraction (with hexane, dichloromethane (DCM) and MeOH) was used to fractionate the components of *A. digitata*, *C. procera* and *E. hirta* into non-polar, medium polar and polar fractions. The yields of 50% aq.MeOH extracts of AD, CP and EH were 8.43, 4.49 and 2.93% respectively. The soxhlet methanol extracts gave yields of 6.17, 4.22 and 3.42% for AD, CP and EH respectively. SPE cleanup of the methanol extracts gave total percentage yields of 68.43, 45.59 and 56.83% for AD, CP and EH respectively. The extracts and fractions were characterised and some functional groups present in them obtained.

3. Determination of the *in vitro* antioxidant, anti-inflammatory and antibacterial activities of the 50% MeOH and soxhlet extracts.	The *in vitro* antioxidant, anti-inflammatory and antibacterial activities of the extracts and fractions were determined. The percentage inhibition concentrations were also obtained for all the extracts and fractions investigated and IC_{50} calculated for those with up to 50% minimum inhibition. The extracts and fractions with the highest antioxidant activities were: from 50% aq.MeOH extracts; AD(EtOAc) = 88.90% (IC_{50}: 3.476 x 10^{-2} mg/mL), from soxhlet extracts: AD(MeOH) = 89.63% (IC_{50}:4.02 x10^{-2} mg/mL) and from SPE fractions: $AD(MeOH)_2$ = 94.96% (2.933 x 10^{-2} mg/mL). The anti-inflammatory investigation showed that the highest anti-inflammatory activity was at 100 μg/mL concentration for all the extracts and comparing the plants, the trend observed was AD > EH > CP at the same concentrations. Antibacterial results revealed that seven extracts had activities against *S. aureus* while six had activities against *B. cereus.*
4. Bioactivity-guided isolation of antioxidant compounds from the plant extracts.	A constituent of the EtOAc fraction from AD was chromatographed on HPLC and characterised by physical appearance, melting point, retention time, UV-Vis and mass spectral data and was identified as quercetin.

6.2 CONCLUSION

The findings in this report have established the presence of some bioactive compounds such as phytol, n-hexadecanoic acid, oleic acid, some saturated fatty acids and squalene in the essential oils of some of the plant parts which are used in the management of bronchial asthma in Alternative Medicine. Some of these plants have appreciable antioxidant, anti-inflammatory and antibacterial activities which are relevant in the management of bronchial asthma. This research work also lends support to the use of these plants by practitioners of Alternative Medicine in the management of bronchial asthma.

CHAPTER SEVEN

7.0 CONTRIBUTIONS TO KNOWLEDGE

1. Modified hydrodistillation method involving hourly collection of samples was developed and applied in this study.
2. An antioxidant compound, quercetin, was isolated from *Adansonia digitata* leaves for the first time to the best of my knowledge.
3. *Adansonia digitata* was established to show strong anti-inflammatory, anti-oxidant and antibacterial activities which confirm its potential in the management of asthma.

7.1 FUTURE WORK

Further work relevant to the management of asthma using these plant parts include animal studies and the determination of lethal concentration (LC_{50}) which is the standard measure of toxicity to find out the concentration beyond which there might be apoptosis of cells.

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