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PLANT DERIVED COMPOUNDS AGAINST THE WIDESPREAD WEED:
PARTHENIUM HYSTEROPHORUS

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Plant derived compounds against the widespread weed: *Parthenium hysterophorus*

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APPROVAL SHEET

Addis Ababa University

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This is to certify that this Dissertation prepared by Zelalem Gizachew, entitled: plant derived compounds against the widespread weed: *Parthenium hysterophorus*, submitted in partial fulfillment of the requirement of the degree of Doctor of Philosophy in Chemistry (Organic stream) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION OF THE CANDIDATE

I, hereby, declare that this submitted for the degree of Doctor of Philosophy (PhD) in Chemistry (Organic stream) at Addis Ababa University, College of Natural and Computational sciences, Addis Ababa, Ethiopia, is my own original work and that it has not been submitted previously to any institution or higher education. All sources of materials used in this work have been duely acknowledged.

This work was done from September 2013 to March, 2019 at Addis Ababa University College of Natural Sciences, Department of Chemistry and Ambo Agricultural Research Center.

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List of acronyms and Abbreviations

APPRC	Ambo Plant Protection Research Center
Amh	Amharic
ABD	Average Dry Biomass
CC	Column Chromatography
GDP	Growth Domestic Product
GI	Growth Inhibition
h	hour
HPLC	High Performance Liquid Chromatography
IR	Infrared spectroscopy
IWM	Integrated Weed Management
NaOCl	Sodium hypochlorite
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
m	meter
Km	Kilometer
mg/mL	milligram per millilitre
Lit	Literature
M1	Mean1
M2	Mean 2
MIC	Minimum Inhibition Concentration

NMR	Nuclear Magnetic Resonance Spectroscopy
Rf	Retention factor
T1	Treatment one
T2	Treatment two
T3	Treatment three
Ap	Aerial part
Lf	Leaf
Bk	Bark
Fr	Fruit
Wd	Wood
Rt	Root
UV	Ultraviolet spectroscopy

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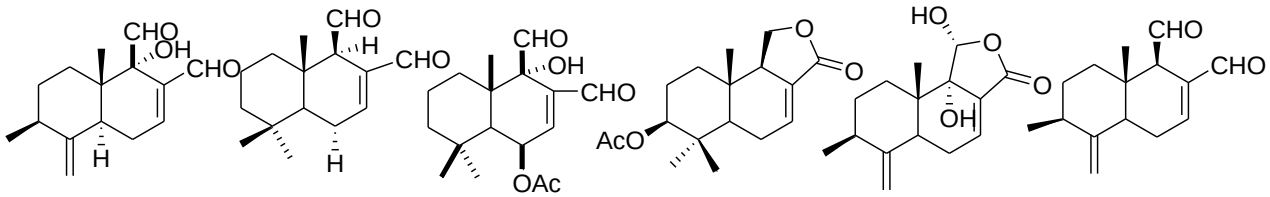
Abstract

Weeds are plants growing in agriculture areas where they are not wanted and gravely affect the agriculture sector more than other crop pests. *Parthenium hysterophorus* (*Kinche arem* in Amharic) is one of the worst invasive weeds in the world. It is poisonous to germination and growth of crops. It is estimated that this weed decreases the production of crops by more than 25%.

To date attempts to control the spread of this weed by synthetic chemicals have not been successful. Allelopathic plants produce natural chemicals that inhibit growth of weeds. In this project we have screened several plant extracts and purified compounds in order to find substances that may be used to combat the spread of the above weed.

In this project we have tested several plant extracts to study their effect on the growth of the above weed. We discovered that the ethanol extracts of *Echinops kebericho* root*, Warburgia ugandensis* bark*,* aerial parts of *Solanecio gigas, Achyranthes aspera* and *Premna schimperi* completely inhibited Parthenium seed germination at a dose of 0.05 mg/mL. Using bioassay-guided protocol; dehydrocostus lactone (**28**) isolated from the root of *E. kebericho* was found to be the most active. Muzigadial (**48**) and cinnamolide-3β-acetate (**64**) from *W. ugandensis* bark were also very active. In the course, albicanyl acetate (**115)** and caseamemin (**117**) were isolated for the first time from this plant species with other known compounds like ugandensidial (**60**), polygodial (**49**), 9-deoxymuzigadial (**116**), and 11α-hydroxymuzigadiolide (**66**) with moderate phytotoxic activities. Other compounds with intermediate activities were also isolated from *A. aspera* (stigmasterol glycoside (**82**) and *P. schimperi* (kaempferide (**112**)).



1. Introduction

In Ethiopia, the agricultural sector is the cornerstone of the economic and social life of the people. Its economy now depends mainly on agricultural products accounting for about 40 percent of gross domestic product (GDP) [1]. Eventhough crops are the main products of the country to feed its population, the yield loss assessment study indicates that weeds, diseases, and insect-pests are the major causes for the decrease of its production. Among these biotic factors, weeds cause a yield loss of about 25% in the least developed countries. The amount of crop yield losses due to weeds in Ethiopia has not been well documented [2].

Weeds are a menace to all crop types. They are known to compete for light, water and nutrients and harbor diseases and insects [3]. *Parthenium hysterophorus* L. (Figure 1) also known as Peterson's weed or Santamaria feverfew is one of the devastating and aggressive annual herbaceous weeds. This weed ia now called kinch Arem in Amharic. It is allelopathic, competitive and poisonous for plant germination and growth [3].

Figure 1. *P. hysterophorus* weed (Ambo, APPRC garden Ethiopia)

P. hysterophorus weed is now considered as a dominant weed and considered as the worst and most frequent weed species in areas of eastern, central and northern Ethiopia [2]. The weed has high reproductive and disseminative potential and spread in alarmingly fast rate to invade any environment. It is now found in large areas of Ethiopia. Sustainable

production of many crops is at risk in areas invaded by this weed. The weed causes yield losses of up to 40% to several crops [4]. It also damages many crops by using as an alternative host for many crop pests.

Moreover *Parthenium* weed has also adverse effects on both human and animal health [4, 5]. It is known to cause asthma, bronchitis, dermatitis and hay fever in humans. Previous chemical investigation indicates that the plant contains sesquiterpene lactones and different phenolic acids [6]. These compounds are known be harmful to human beings and animals [7]. Allelopathy refers to the detrimental effect of one plant species on seed germination, growth and reproduction of another plant species. The allelopathic effects of this weed is due to the presence of these compounds [7].

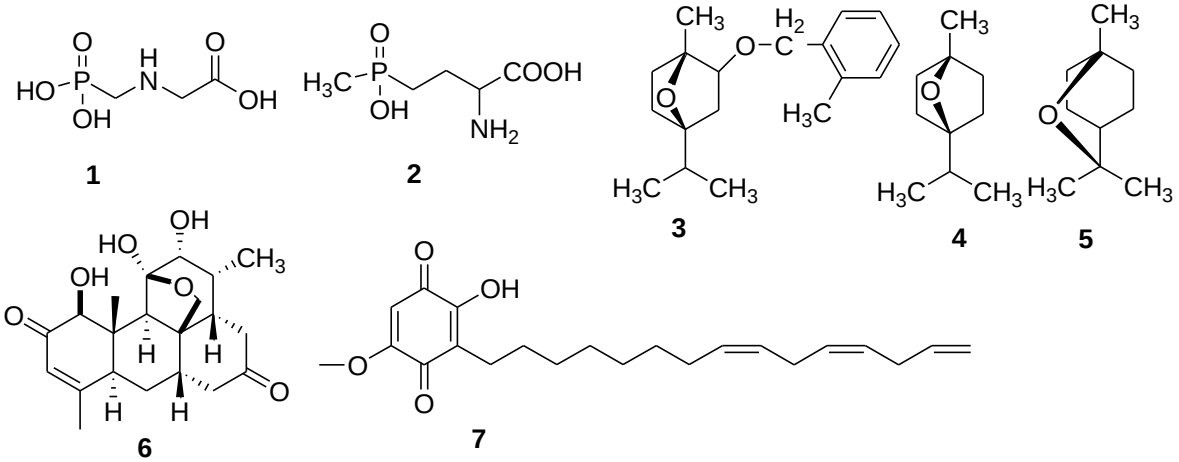
Study about the controlling mechanism of this weed should be a great concern for the food security of Ethiopian people. Attempts to control the spread of the weed using synthetic chemicals and biological controlling agents have so far not been successful.

On the other hand, its rapid spreading has become a bigger threat to the reduction of the production and the quality of many crops. So, safe and effective controlling methods should be applied to increase crop productivity.

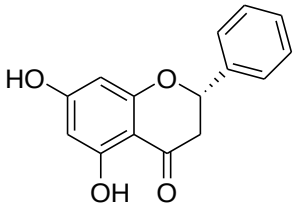
The bulk of past weed control trials in Ethiopia relied heavily on the use of synthetic herbicides such as roundup®, glyphosate (**1**) is the active ingredient, which is a broad spectrum, non-selective, systemic herbicide. Although synthetic herbicides have played a significant role, their excessive use has have caused various problems such as (i) pollution and contamination of the environment, ii) tissue deterioration in treated area, (iii) growth inhibition, (iv) foliar chlorosis and necrosis and (v) detrimental effects to non-target beneficial crops, and microorganisms [8]. As a result, alternative weed control methods have been tried including (i) mechanical control (ii) hand weeding (iii) crop rotation, and (iv) allelopathic botanicals. Hand weeding mostly used by small-scale farmers is more difficult due to the allergic effects of parthenium on the human body. Mechanical control, on the other hand, is costly in terms of machinery, labor and time requirement. Farmers who are poor in resource may not afford the purchase of herbicides in addition to its effects on the human health and environment [9].

The introduction of herbicides in organic agriculture is a welcome move in order to control obnoxious weeds and thereby reduce yield loss. Hence, research should be done to find out some natural way to minimize the dependency on synthetic herbicides. Natural products are attractive alternatives since they are environmentally friendly and easily biodegradable.

Phosphinothricin(**2**), the biosynthetic version of glufosinate and bialaphos, is a commercial phytotoxic microbial product obtained from *Streptomyces viridochromogenes* [10-12]. Kohli *et al* reported that eucalypt oils extracted from *E. globulus* and *E. citriodora* showed phytotoxic impacts on *P.hysterophorus* weed by reducing seed germination, chlorophyll content and cellular respiration significantly [13]. Cinmethylin (**3**), a commercial herbicide, is a 2-benzyl ether-substituted analog of the natural product 1,4-cineole (**4**) and has been sold in Europe and Asia. This compound breaks down to give rise to a true herbicide, 2-hydroxy 1,4-cineole [14]. 1,8-cineole (**5**) and it's natural analog 1,4-cineole (**3**) obtained in essential oils of many plants. Both suppress the growth of several weeds [15]. Hailanthone (**6**), a chaparrinone-type quassinoid, has broad weed spectrum activity when applied either as preemergence or postemergence herbicide and provides 100% weed control of green foxtail (*Setaria viridis*) and sicklepod (*Cassia obtusifolia*) at rates of 0.125 kg/ha) [15]. Another successful example of the study of plant-plant interaction resulted in the discovery of a potent phytotoxin sorgoleone (**7**) from Sorghum species [16].



Generally, natural herbicides with new modes of action are needed due to the rapidly evolving resistance of weeds like parthenium to commercial herbicides [17]. For instance, pinocembrin (**8**), isolated from *Flourensia oolepis,* is phytotoxic and might offer new and effective tools in organic agriculture [18] Duke et al. (1987) first reported the phytotoxic activity of artemisinin (**9**) isolated from *Artemisia annua* with action of disrupting the formation of microtubule organizing centers, may be attributed to loss of membrane integrity [15, 19].



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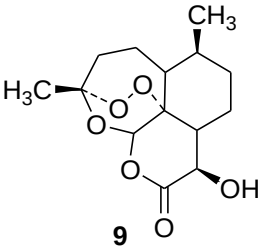

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Therefore, there is a continuing need to develop herbicides from natural origin which are biodegradable, hence, has less negative impact on the environment [20, 21]. Hence it is important to research on plants that have herbicidal properties followed by isolation and characterization of the bioactive ingredients.

1.1. What are invasive weeds?

Weeds are undesired plants that grow in the natural environment and interfere with crops and human activities. Weeds are considered as the most underestimated pests in tropical agriculture. They affect human activities more than other crop pests. Although crop yield losses due to weeds vary from crop to crop and from region to region, because of various biotic and abiotic factors, it has been estimated that weeds cause a yield loss of about 10% in the less developed countries and 25% in the least developed countries [2]. There are many grass weeds and broad-leaved weeds that seriously affect the production of cereals and pulses. *Parthenium hysterophorus* is one of the toxic and devastating weeds of global concern.

1.1.1. *Parthenium hysterophorus* as a major weed

Parthenium hysterophorus L.; commonly named (Eng.): Bitterweed, false ragweed, feverfew, Parthenium weed, Ragweed, white top, carrot weed, Santamaria weed, etc. It is an annual, erect and profusely branched herb with an average height of 50-150 cm.

Parthenium hysterophorus L. (Asteraceae) is native to tropical America and has spread to Asia, Africa, Australia, and Europe. It is among the top ten worst weeds of the world and has been listed in the global invasive species database due to its prolific seed production. A typical mature plant produces up to 25,000 seeds per a mature plant [22]. Since the weed is highly in need of light, water, and nutrients, it affects the production of many crops. Parthenium is an invasive and destructive weed particularly in Eastern part of Ethiopia. It is noxious because it is highly adaptable to almost all type of environmental conditions, can invade all types of land, and also causes high losses in the yield of field crops up to 40% [23].

Furthermore, seeds germinate throughout the year given suitable moisture levels and remain viable for a long time and thrive under very harsh environmental conditions. The weed also has a very high regenerative potential to adapt to any adverse environmental conditions. Under drought stress the weed can germinate, grow, mature and set seeds in 4-weeks. Its invasive nature is evident from its ability to form huge monocultural stands with no other plant in the vicinity. It causes numerous environmental and agricultural problems (loss of crop productivity, fodder scarcity, biodiversity depletion) and health problems for human beings and livestock. Changes in blood chemistry and inhibition of liver dehydrogenases, as well as degenerative changes to liver and kidney, have been reported in buffalo and sheep [24, 25].

Parthenium hysterophorus has many host ranges and as a result, can be taken as a serious threat to the ecosystem as a whole. Crops most affected are wheat, barley, teff, coffee, okra, sorghum, maize, millets, chickpea, lentils, etc.

1.1.2. Distribution of *P. hysterophorus* in Ethiopia

It is reported that *P. hysterophorus* was first introduced to Ethiopia during the Ethiopian-Somali war in the 1970s and specifically observed around Dire Dawa [26, 27]. Its

introduction was via army vehicles during the war or along with contaminated grain in the course of food aid from western countries to the people who suffered by the war. Since then it has spread to several parts of the country in alarming proportion, through vehicular traffic, wind, water, and urban waste. The recent studies have indicated that African countries are at high risk of invasion [27].

1.1.3. Yield loss of crops caused by *Parthenium hysterophorus*

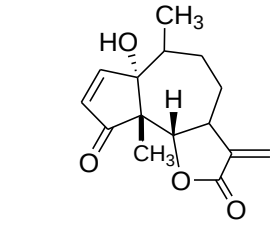
The allelopathic properties of the weed inhibit seed germination of cereal pulses. It was reported that severe crop losses due to parthenium weed in the least developing countries like Ethiopia are around 25% [2, 28]. Sorghum grain yield losses between 40 and 97% were reported in Ethiopia if parthenium is left uncontrolled [23]. Accumulation of Parthenium pollen clusters on floral parts of maize cause 50% reduction in grain filling [29].

Weeds are notorious yield reducers that are, in many situations, economically more important than insects, fungi or other pest organisms. The spreading mechanism of the weed is due to the seeds mainly dispersed through water currents, livestock and other animals, and the movement of vehicles, machinery, grain, stock feed and to a lesser extent by wind. Most of the long-distance spread is through vehicles, farm machinery, and flooding. The spread of seeds plus and their ability to remain viable in the soil for many years pose one of the most complex problems for control [24]. Seeds do not have a dormancy period and are capable of germinating anytime when moisture is available.

1.1.4. Effects of *Parthenium hysterophorus* on humans and animals

The chemical studies of *P. hysterophorus* resulted the isolation of a toxic sesquiterpene lactone, parthenin (**10**) as a major constituent [30] and other phenolic acids such as caffeic acid (**11**), vanillic acid (**12**), ferulic acid (**13**), p-anisic acid (**14**), p-coumaric acid (**15**), para-hydroxybenzoic acid (**16**) and chlorogenic acid (**17**). Due to these compounds, this weed remained harmful to human beings and animals [29]. Parthenium induces severe dermatitis and often allergic symptoms in human beings and animals [30].

Exposure to the weed may cause hay fever, asthma or dermatitis. A hydroxyproline-rich glycoprotein is the major allergen in *P. hysterophorus* pollen [31].



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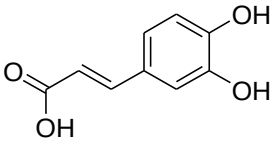

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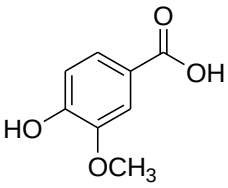

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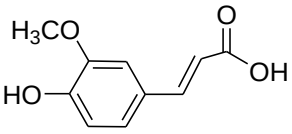

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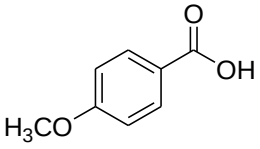

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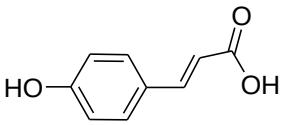

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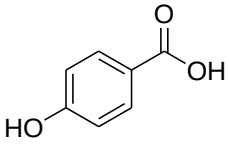

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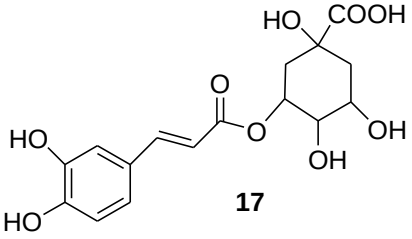

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1.1.5. Controlling options of *Parthenium hysterophorus* weed

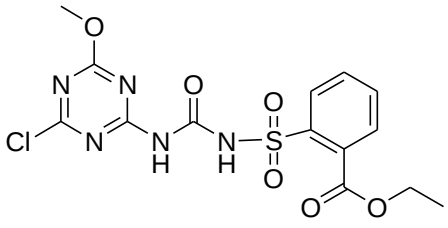
The problem of weed control is often cited as one of the major constraints to organic crop production [32]. The following weed management strategies have been used so far.

1.1.5.1. Mechanical controlling method

The most widely employed controlling practice by farmers to manage the weed in agricultural land is using mechanical controlling method. Uprooting the weed manually by farmers is commonly used but this is difficult due to the allergic effects of parthenium on the human body. Mechanical controlling of weeds is one of the options, it is difficult and met with limited success due to the high cost of machinery, labor and time requirement.

1.1.5.2. Using synthetic chemicals

Using synthetic chemicals like glyphosate (**1**), chlorimuron ethyl (**18**), atrazine (**19**), ametryn (**20**), bromoxynil (**21**), and metasulfuron (**22**) as a weed controlling strategy is widely used in the world. But, their serious effects on the environment and human health lead them not to be the first candidate to manage parthenium weed. In addition to this, developing resistance of weeds to synthetic chemicals and their high cost remain as challenges to using these chemicals as weed control tools.



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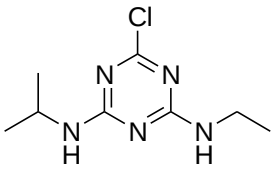

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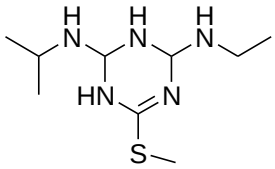

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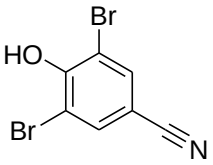

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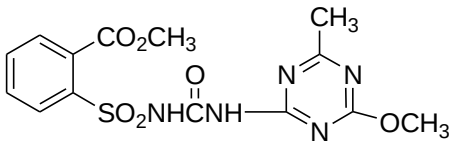

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1.1.5.3. Biological controlling agents

Biological control is the utilization of organisms like bacteria, fungi, insects, etc for the regulation of weeds [33]. Especially the use of rusts in controlling weed was found promising [33, 34]. In this method, microorganisms may kill the weed by the toxins they produced the so-called bioherbicides. In addition to microorganisms, the use of insects like leaf-feeding beetles (*Zygogramma bicolorata*) and a stem-boring weevil (*Listronotus setosipennis*) as a natural enemy may help to control spread of parthenium weed [24, 29, 35]. Predators, pathogens, and parasites serve to keep other organisms population density at a lower average level than would occur in their absence. There are two approaches used in biological control: Classical biological control and non-classical biological control [36]. The former involves the introduction of natural enemies from their native range into an exotic range where their host plant has become a weed. This is an approach most commonly implemented as a biological controlling strategy. The later concentrates on the use of native natural enemies as inundated (release of large numbers of the agent to control the target weed, e.g. mycoherbicides).

But, biological control method is only successful in a greenhouse and small farming areas due to the difficulty of getting lots of insects and microorganisms on large farms [33]. These biological agents may also have a broad host range and hence may damage other beneficiary crops and as a result, it is not taken as the first choice of controlling strategy.

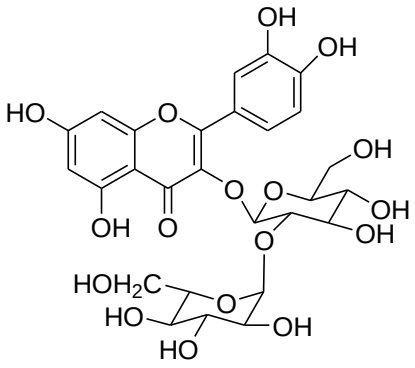
1.1.5.4. Using natural products for weed control

Allelopathic plants and their by-products for weed control is an attractive alternative. It creats a center of attention on organic agriculture and hence considered as the future generation of herbicides. This is because natural products are less toxicity to other beneficiary microorganisms, environmentally friendly, easily biodegradability than synthetic compounds [37-39].

The term allelopathy is made up from two Greek words ‘allelon’ meaning “each other” and ‘pathos’ meaning “suffering”. It describes positive (sympathic) and negative (pathetic) interactions of plants [21]. Release of chemicals from a plant to the environment may act as a growth promoting or growth inhibiting to another plant in the vicinity. But, ecologists also favor a negative definition that defines allelopathy as growth suppression of one plant species by another due to release of toxic compounds known as allelochemicals. These compounds play important roles in the determination of plant diversity, dominance, succession, and productivity of agroecosystems [40]. Allelopathy is a difficult phenomenon to study due to the fact that seed germination inhibition and plant growth suppression may also be due to the adverse effects of plant residues that lead to immobilization of large amounts of nutrients by micro-organisms involved in decomposition [41]. It was first reported in 1966 that a wasteland weed, *Xanthium strumarium*, compete with Parthenium. Another report in 1983 stated that *Cassia sericea* suppress Parthenium weed significantly to be used as biological controlling tool [35]. Then numerous plants and living organisms are reported to possess allelopathic potential and efforts have been made to use them in weed control [42]. Many of the secondary products of living organisms have evolved in response to biotic interactions. Thus, plant derived compounds sometimes have some type of biological activity at lower concentrations than compounds derived from chemical synthesis programs [10].

Attempts were made by different scholars to control parthenium weed using plant-based herbicides. For instance, compounds isolated from the leaves of *Mangifera indica* exhibited herbicidal activity against *Parthenium hysterophorus* weed [43]. Quercetin-3-O-glucopyranosyl-(1, 2)-D-glucopyranoside (**23**) was the most active and could be used as herbicide at 15% concentration. The other compound, 6-(p-hydroxybenzyl) taxifolin-7-O-

D-glucoside (tricuspid) (**24**), caused yellowing of parthenium seedlings at the same concentration.



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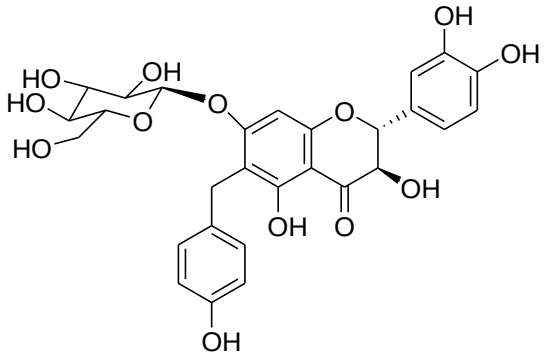

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The root and shoot aqueous extracts of three kinds of grass (*Dicanthium annulatum, Cenchrus pennisetiformis,* and *Sorghum halepense*) were tested against *Parthenium hysterophorus* seed germination at different concentrations and the highest seed suppressive was exhibited by aqueous extracts of *C. pennisetiformis* [44]. Knox, 2013 studied that Parthenium seed germination was inhibited with aqueous foliar extracts of *Cassia occidentalis, Andrographis paniculata, Abutilon indicum* and *Hyptis suaveolens* [39].

In the previous effort made to combat *Parthenium hysterophorus* weed by Knox *et al.* 2010, the shoot aqueous extracts of *Azadirachta indica, Solanum nigrum, Calotropis procera, Cymbopogon citrates, Withania somnifera, Cassia occidentalis* and *Ocimum sanctum* were studied at 50 and 100% concentrations. It is reported that maximum seed germination inhibition was observed for *Cassia occidentalis* [45]. Another *Cassia uniflora* leachat extracts significantly inhibited the germination of Parthenium seeds [46].

1.1.5.5. Techniques of allelopathy

Plant allelopathy is one of the modes of interaction between receptor and donor plants [47]. It is a common biological phenomenon by which one plant releases allelochemicals that influence the growth, survival, development, and reproduction of other plants. Allelochemicals can potentially be used as growth regulators, herbicides, insecticides, and antimicrobial crop protection products. These chemicals can be released from one

plant to the other plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems [48]. Rainfall causes the leaching of allelopathic substances from leaves to the ground during period of stress. Root exudation includes the secretion of ions, free oxygen and water, enzymes, mucilage, and a diverse array of carbon-containing primary and secondary metabolites. Roots release these compounds via two potential mechanisms: either by transporting across the cellular membrane or by secreting in to the surrounding rhizosphere [47]. Allelochemicals are released and added to the soil over a time period and immobilised from the soil solution by plant uptake, adsorption to soil particles, and degradation by microorganisms (Figure 2).

The mode of action of allelochemicals and the physiological mechanisms of allelopathy, consisting of the influence on cell micro and ultra-structure, cell division and elongation, membrane permeability, oxidative and antioxidant systems, growth regulation systems, respiration, enzyme synthesis and metabolism, photosynthesis, mineral ion uptake, protein and nucleic acid synthesis [49].

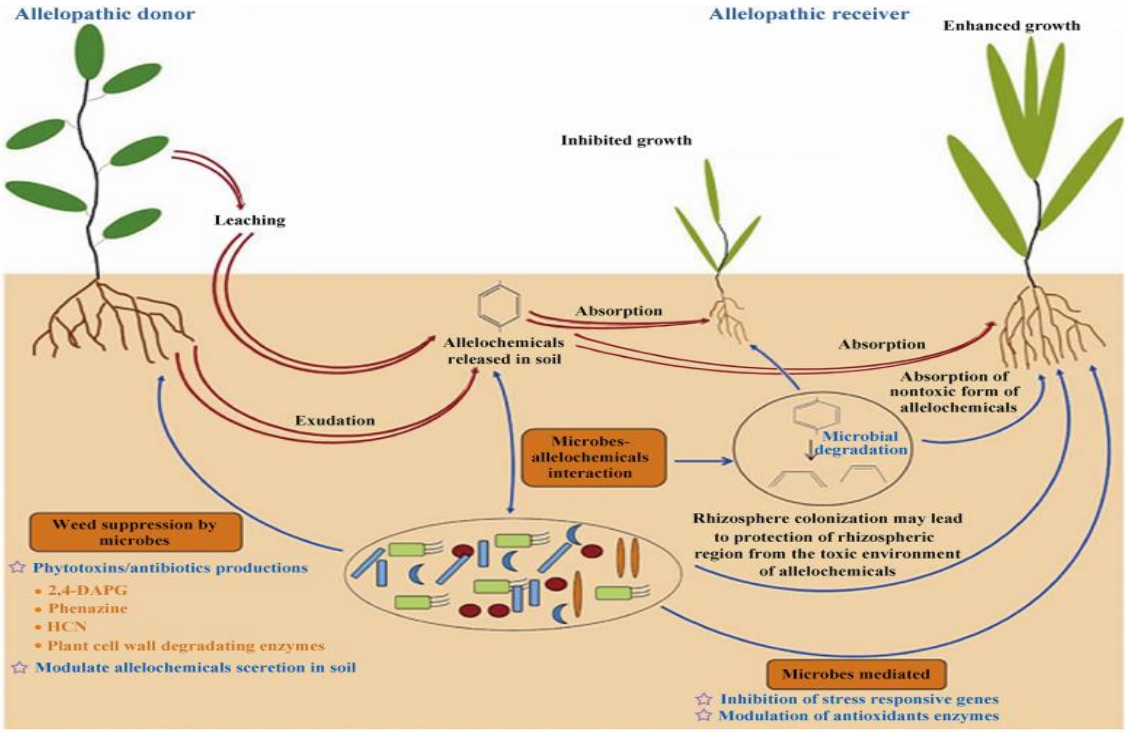


Figure 2. A diagram showing the interactions of allelopathic donor and acceptor plant species [49]

1.2. Berief review of the literature on candidate plants

The broad biological activities of *E. kebericho, W. ugandensis, A. aspera* and *P. schimperi* against different microorganisms reported previously is the reason for the selection of these plants to be studied for their phytotoxic activity against *P. hysterophorus* weed.

1.2.1. *Echinops kebericho*

The Asteraceae is a very large and widespread family of flowering plants with with more than 1,620 genera and 23,600 species distributed throughout the world. Members of this family have flower heads composed of many small flowers. Echinops is a genus of about 120 species of flowering plants in the family Asteraceae, commonly known as globe thistles. They have spiny foliage and produce blue or white spherical flower heads [50-52]. *E. kebericho,* known only in Ethiopia, is an erect massive rootstock-bearing perennial herb or shrub that grows up to a height of 1.2 m with leafy stems. It is among the most important medicinal plants of the country, valued primarily for its root parts [53]. Traditionally the smoke from burning the root (Figure 2) is inhaled to cure diseases like a headache, diarrhea, malaria, taenicide, hemorrhoids, typhus, etc [50, 53, 54]. The root is burned as fumigant, mainly after childbirth, to ward off mosquitoes and as snake repellant [53].

Figure 3. *E. kebericho* root purchased from market

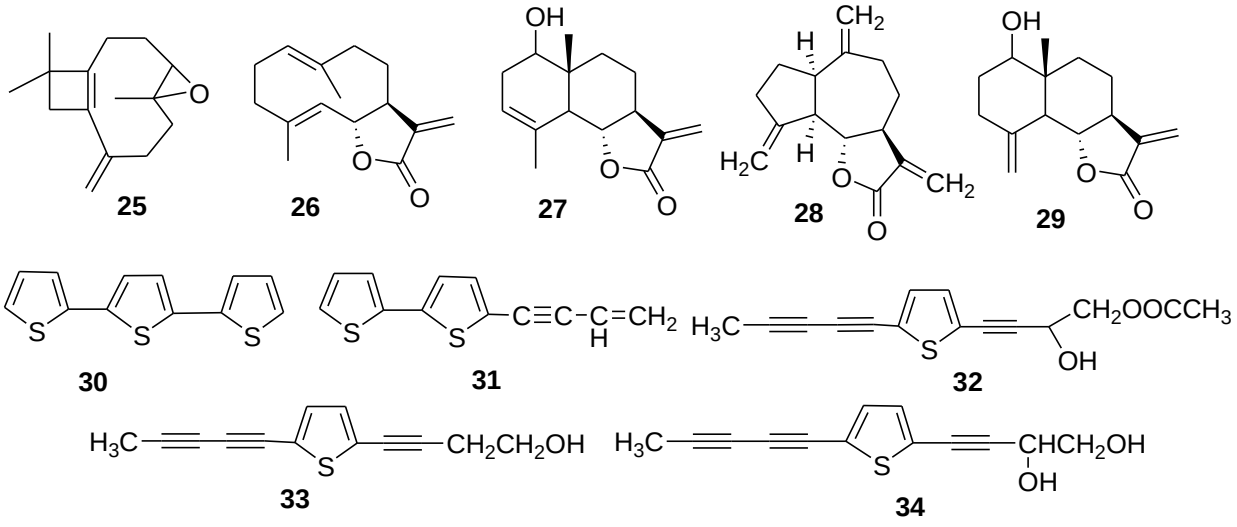
The biological activity studies on the genus *Echinops* showed antibacterial, antifungal and anti-inflammatory properties [55]. The ethanol and methanol crude extracts of the roots of *E*. *kebericho* showed significant antimicrobial activities against *S. aureus, C. albicans,* and *A*. *flavus* using concentrations from 3.12 to 25µg/ml in the disc diffusion assay [55] .

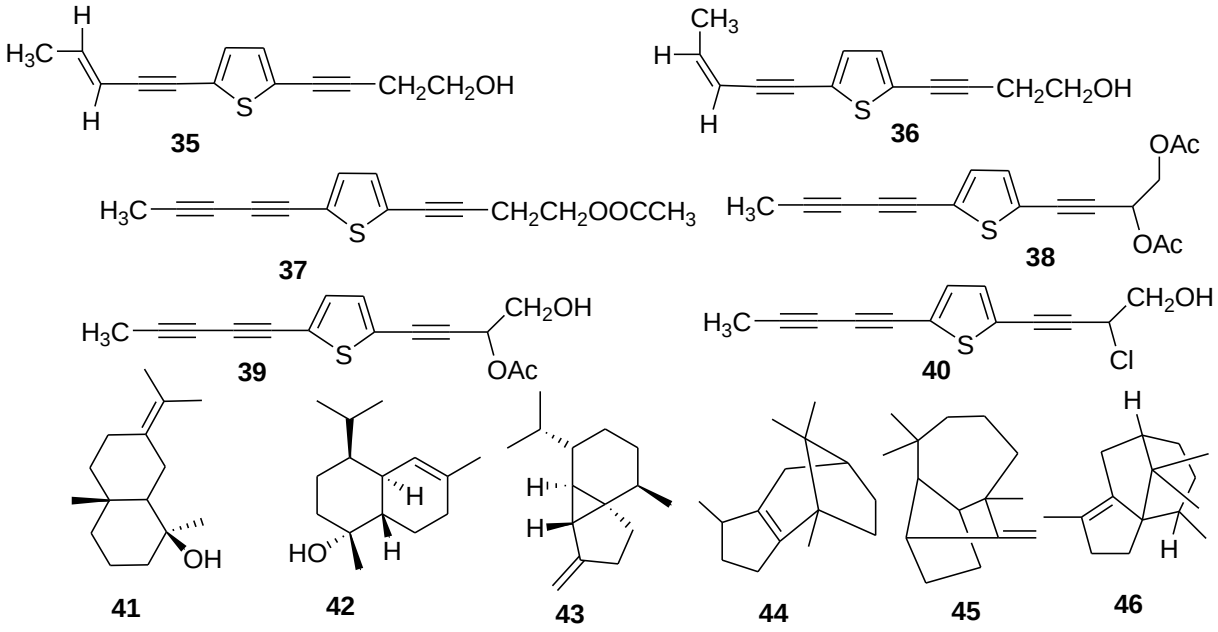
The aqueous root extract of the plant is found to have anti-diarrheal and anti-spasmodic activities [56].

The anti-malarial activity of ethanol extract of *E. kebericho* root against *Plasmodium berghei* was reported and the result was found promising to be used as an alternative to modern synthetic insect/mosquito repellent agents [54, 57].

Based on its biological activity interest, Abegaz, B. *et al*., reported sesquiterpene lactones, polyacetylenes and acetylenic thiophenes from the species of echinops [58]. The study on the endemic species from Ethiopia, *E. kebericho*, gave copious amounts of sesquiterpenes of which dehydrocostus lactone (**28**) is the major one and caryophyllene epoxide (**25**), costunolide (**26**), santamarin (**27**), reynosin (**29**) and thiophene derivatives (**30**-**40**) [58].

Different essential oils were identified from volatile fractions of roots of *Echinops kebericho* Mesfin obtained by hydrodistillation and solidphase microextraction (SPME). The main constituent found in hydrodistilled essential oil was eudesm-7(11)-en-4-ol (**41**), followed by caryophyllene oxide (**25**) and τ-cadinol (**42**). High amounts of β -cubebene (**43**), followed by β-patchoulene (**44**), longifolene (**45**), and cyperene (**46**), were detected by SPME [59].





Dehydrocostus lactone (**28**), obtained from ethyl ether fraction of *n*-hexane extract of *Saussurea lappa* root, was found phytotoxic to inhibit the growth of two kinds of grasses and two broadleaf plants with greater than 85% growth inhibition [60]. The mechanism of action of this compound was found inhibition of fatty acid synthesis which in turn caused weak cell membrane integrity.

1.2.2. Literature review of *Warburgia ugandensis*

The Canellaceae is a family of flowering plants in the order Canellales with has 6 genera and 16 species. The genus *Warburgia* (Canellaceae) includes four species: *W. ugandensis* Sprague, within eastern African highlands, *W. salutaris* Berto f. Chiov found in southern Africa (Lesotho, South Africa, Swaziland, Mozambique, and Zimbabwe), *W. elongate* Verdc. (endemic to Tanzania) and *W. stuhlmannii* Engl. found along the East African coastline [61]. *Warburgia* species are characterized by aromatic and pungent bark, which is used medicinally. *W. ugandensis* Sprague, (Figure 3) is an erect tree evergreen, single stem aromatic perennial plant whose height ranges between 4.5 and 30 m with a diameter of up to 70 cm or more. The tree is one of the priority species with multifunctions; used in the extraction of herbal medicine, as timber, poles, and charcoal burning, firewood, tool handle, for soil conservation, ornaments, shade, and resin [62].

Figure 4. *W. ugandensis* (bark and leaves) collected from Bale Zone, Ethiopia

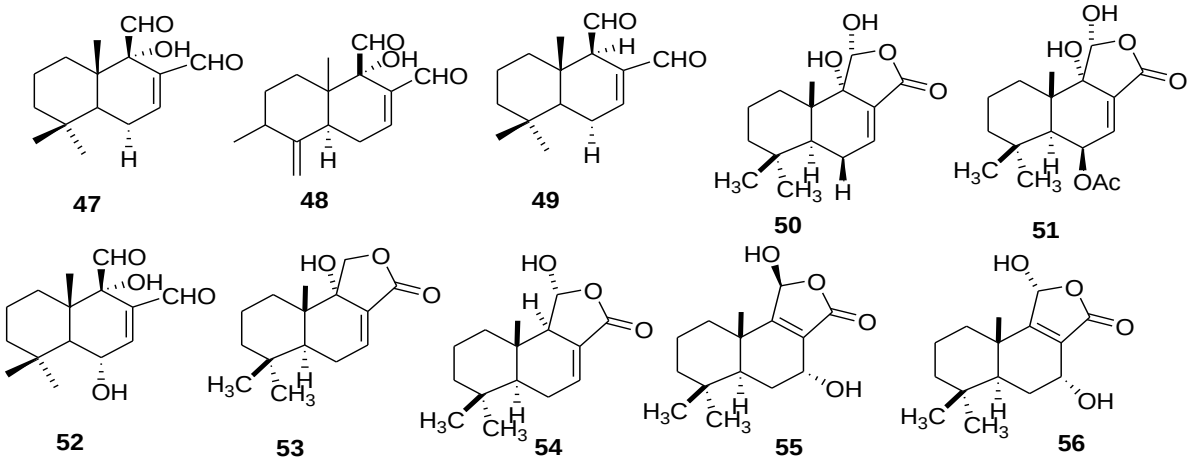
Traditionally, *W. ugandensis* (Canellaceae) is used as a remedy for stomachache, constipation, toothache, and malaria [63, 64]. It is also used for the treatment of sexually transmitted diseases, diarrhea, cough and internal wounds/ulcers [65, 66]. All parts of the plant have a hot peppery taste. Leaves and seeds are used to add flavor to curries [62].

On the bases of the indigenous knowledge claimed, a number of Drimane-type sesquiterpenes and flavonoids have been reported from different parts of the plant [67-71].

In vitro pharmacological studies on this plant have confirmed the presence of insect antifeedant [67], molluscicidal [72, 73], antimicrobial [63, 70] and antileishmanial [74] sesquiterpenes. Both *W. ugandensis* and *W. stuhlmannii* bark extracts possessed strong antimicrobial activities against gram-positive bacteria, yeasts and filamentous fungi [75, 76].

Regarding of these wide biological activities, the plant is considered as an important natural African antibiotic and have been used extensively as part of traditional healing practices for the treatment of fungal, bacterial and protozoal infections in both humans and animals [77]. Therefore, chemical investigation of the plant has been done exhaustively due to the above mentioned multidimensional medicinal aspect of drimane sesquiterpenoids. This resulted in the isolation of widely applicable dialdehydic compounds warburganal (**47**), muzigadial (**48**) and polygodial (**49**) [67, 78-80]. The first two compounds are potent helicocides (snail-killer).

It is reported that EtOAc extract of *W. ugandensis* bark exhibited potent cytotoxic activity on the KB cell line (99% and 64% inhibition at 10 and 1 µg/mL, respectively) [81]. Following the activity, different sesquiterpene compounds were isolated using different chromatographic and spectroscopic methods. These are ugandenial A (**50**), 9α, 11α-dihydroxy,6β-acetyl-cinnamolide (**51**), warburganal (**47**), polygodial (**49**), mukaadial (**52**), 9α-hydroxycinnamolide (**53**), and dendocarbins A, L, and M (**54, 55** and **56**). Of the compounds reported, warburganal (**47**), muzigadial (**48**) and polygodial (**49**) were exhibited strong cytotoxic activity against KB cell line [81, 82].

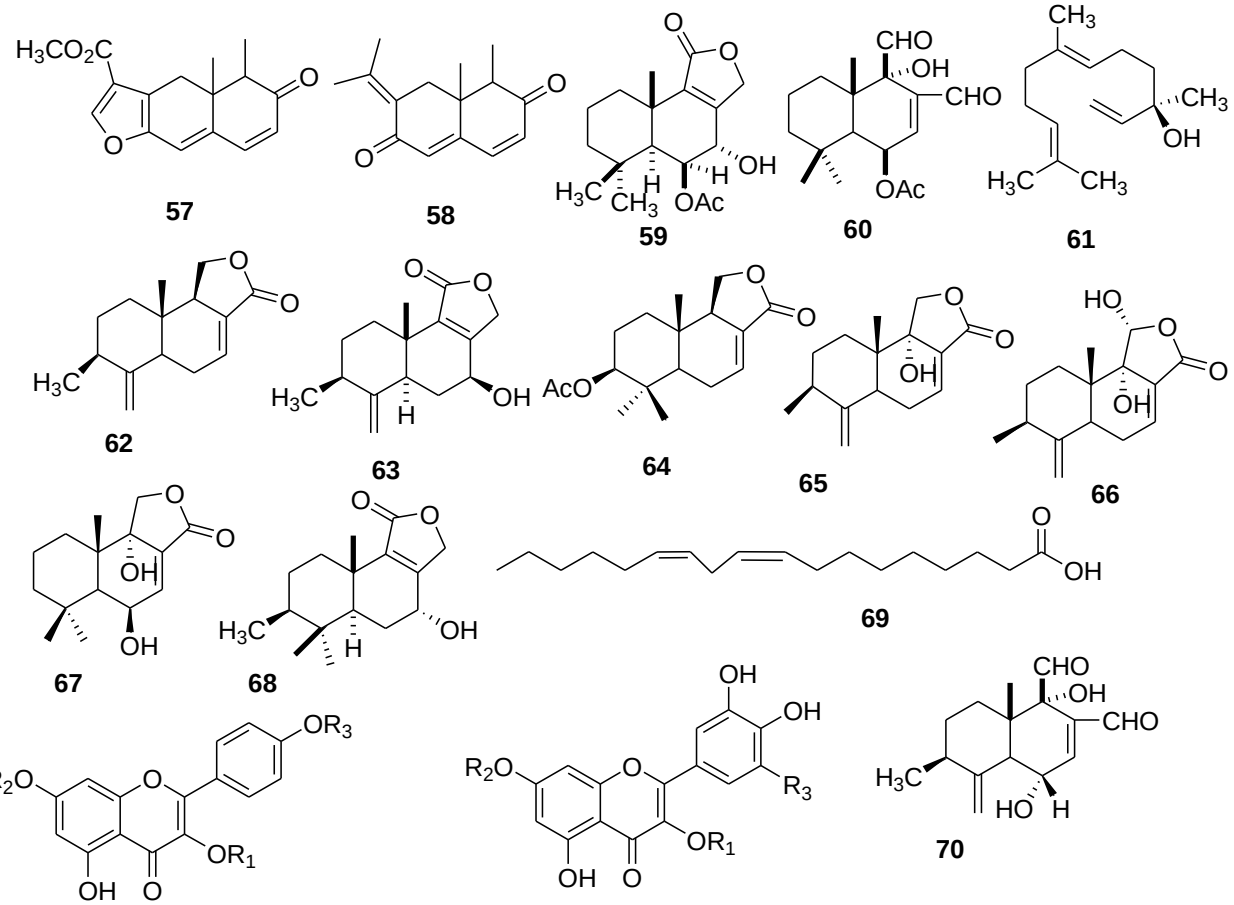


The first phytochemical investigation of *W. ugandensis* led to the isolation of drimane type sesquiterpenoids from the heartwood of the plant. Before that, there was no report on the chemical investigation from the plant except for an early description of the essential oil derivative from the bark of *W. stuhlmanii* [68, 69]. The first reported Drimane-type sesquiterpenoid compounds from the heartwood of *W. ugandensis* are warburgin (**57**), warburgiadione (**58**), ugandensolide (**59**) and ugandensidial (**60**).

A similar phytochemical investigation work on stem bark of *W. ugandensis* was done in Addis Ababa University by Gonfa, T. and four sesquiterpene compounds namely nerolidol (**61**), muzigadial (**48**), ugandensidial (**60**) and cinnamolide-3β-acetate (**64**) were reported [83].

Antibacterial sesquiterpenoids were isolated from *W. salutaris* and *W. ugandensis* against *Mycobacterium aurum, M. fortuitum, M. phlei* and *M.smegmatis* in which linoleic acid showed higher activity than other sesquiterpenoids [70, 84]. The compounds isolated from *W. ugandensis* in the course of studying with antibacterial activity are 4(13),7-coloratadien-12,11-olide (**62**), and 7β-hydroxy-4(13),8 coloratadien-11,12-olide (**63**), together with nine known sesquiterpenes, i.e., cinnamolide-3β-acetate (**64**), muzigadial (**48**), muzigadiolide (**65**), 11α-hydroxy muzigadiolide (**66**), pereniporin-B (**67**), 7α-hydroxy-8-drimen-11,12-olide (**68**), 6α,9α-dihydroxy-4(13),7-coloratadien 11,12-dial (**69**), and linoleic acid (**70**) [70, 84, 85].

Flavonol glycosides (**71-75**) together with known flavonols like kaempferol (**76**), kaempferol-3-rhamnoside (**77**), kaempferol-3-glucoside (**78**), myricetin (**79**), quercetin (**80**) were reported from the leaf methanol extracts of *W. stuhlmannii* and *W. ugandensis* [86, 87].



71 R_1 = xylosyl (1→ 2) glucose, R_2 = H, R_3 = Me
72 R_1 = rhamnose, R_2 = R_3 = glucose
73 R_1 = R_2 = R_3 = glucose
76 R_1 = R_2 =R_3 = H
77 R_1 = rhambose,R_2 =R_3 = H
78 R_1 = glucose, R_2 =R_3 = H

74 R_1 = [rhamnosyl (1→ 6)] [glucosyl (1→ 2)]-glucoside, R_2 =rhamnose, R_3 = H
75 R_1 = H, R_2 = rhamnose, R_3 = H
79 R_1= R_2 =H, R_3 =OH
80 R_1= R_2 = R_3 = H

1.2.3. Literature review of *Achyranthes aspera*

Amaranthaceae is the family of flowering plants with about 175 genera and more than 2,500 species. Most of this families are herbs and subshrubs that distributed nearly worldwide.The genus *Achyranthes* has reported having seven species [88]. These are *A. aspera, A. atollensis, A. bidentata, A. canescens, A. japonica, A. splendens* and *A. mutica. Achyranthes aspera* Linn. (Figure 4) is distributed as a weed up to an altitude of 2500 m in many regions of Ethiopia. *A. aspera* (Telenji in Amharic) is used in the indigenous medical system for acute febrile illness, wound dressing, tonic, diuretic, expectorant, and various ailments [89].

The plant is used by traditional healers against asthma, cancer, leprosy, fistula, arthritis, dandruff, hepatitis, renal disorders bronchitis, cold, cough, colic, debility, dog bite, dysentery, headache, pneumonia, renal complications, snake bite, and skin diseases etc [90, 91].

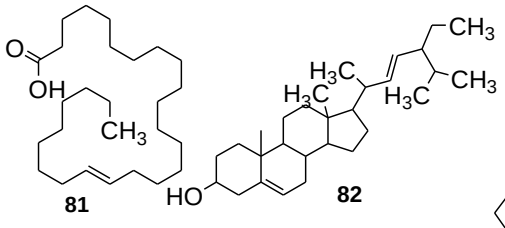
Figure 5. *A. aspera* aerial part (AAU, college of natural sciences, ALNAP garden, Ethiopia)

The root extract has been reported to possess spermicidal activity in human and rat sperm and anti-inflammatory activity [90]. The methanolic extract of the leaves displayed anti-depressant effect in mice and rats. It showed cardiovascular activity due to water-soluble alkaloid, achyranthine (**84**), which decreased blood pressure and heart rate, dilated blood vessels and increased the rate and amplitude of respiration in dogs and frogs [92].

The aqueous leaf extract of this plant showed anticataract, antifungal and antioxidant activities [93, 94]. This extract was phytotoxic to pearl millet (*Pennisetum americanum* L.),

sorghum (*Sorghum bicolor* L.), and guar (*Cyamopsis tetragonolobus* L.) at the concentration of 5%. This phytotoxicity result of *A. aspera* aerial part crude extract was claimed due to the presence of acids reported from the plant like vanillic acid (11), ferulic acid (12) and p-coumaric acid (14) [95].

Further compounds isolated from this plant are n-hexacos-14-enoic acid (**81**), stigmasterol (**82**), trans-13-docasenoic acid (**83**), achyranthine (**84**), edysone (**85**), *β*-sitosterol (**86**), triacontanol (**87**), pentatriacontane (**88**), 17-pentatriacontanol (**89**), octyloctadec-9-enoate (**90**), heptacosan-14-β-ol (**91**), *trans*-13-docasenoic acid (**92**), *n*-hexacos-14-enoic acid (**93**), *n* hexacosanyl-*n*-decaniate (**94**), *n*-hexacos-17-enoic acid (**95**), *n*-hexacos-11-enoic acid (**96**) etc. Two new bisdesmosidic triterpenoid saponins (**97** and **98**) were reported from the MeOH extract of the aerial parts of *Achyranthes aspera* Linn [89, 96, 97].



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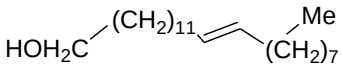

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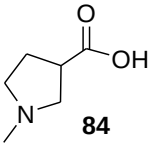

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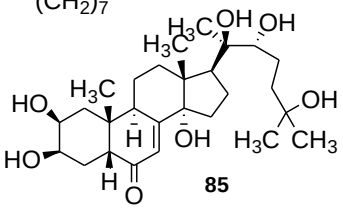

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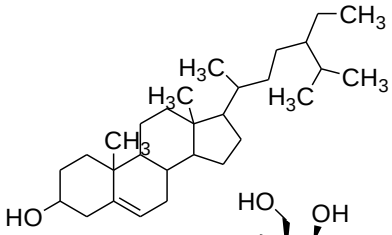

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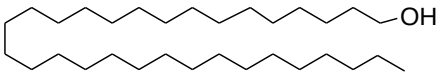

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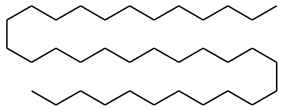

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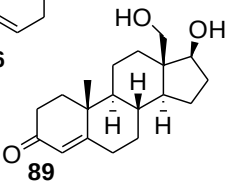


89

$CH_3(CH_2)_7CH=CH(CH_2)_7\text{-}CO\text{-}O\text{-}(CH_2)_7CH_3$

90

$CH_3(CH_2)_{12}\text{-}CHOH\text{-}(CH_2)_{12}\text{-}CH_3$

91

$CH_3(CH_2)_7CH=CH(CH_2)_{11}\text{-}COOH$

92

$CH_3(CH_2)_{10}CH\text{-}CH(CH_2)_{12}\text{-}COOH$

93

$CH_3(CH_2)_8CO\text{-}O\text{-}CH_2\text{-}(CH_2)_{24}\text{-}CH_3$

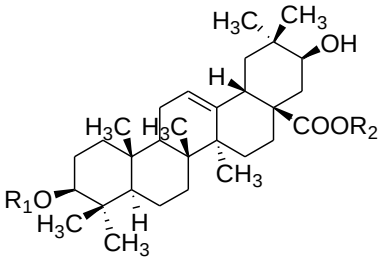
94

$CH_3(CH_2)_7\text{-}CH=CH\text{-}(CH_2)_{15}\text{-}COOH$

95

$CH_3(CH_2)_{13}CH=CH(CH_2)_7\text{-}COOH$

96



97 β-D-glucopyranosyl3β-[O-α-L-rhamnopyranosyl- 1- 3)O-β-D-glucopyranuronosyloxy] machaerinate

98 β-D-glucopyranosyl3β-[O-β-D-galactopyranosyl-1-2)O-α-D-glucopyranuronosyloxy] machaerinate

1.2.4. Literature review of *Premna schimperi*

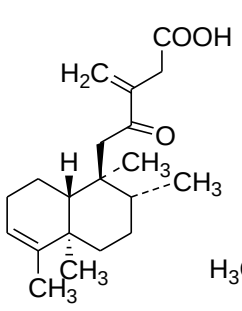
The genus *Premna* which belongs to the family Lamiaceae comprises about more than 200 known species native mainly to the tropical and subtropical Asia, Africa, Australia, and the Pacific islands [98]. A phytochemical review of the literature indicates the genus *Premna* to be a rich source of the iridoid glycosides, diterpenoids, and flavonoids. Furthermore, other classes of secondary metabolites like sesquiterpenoids, triterpenoids, isoflavones, lignans, and xanthones are known to be isolated from different species of *Premna* genus.

Premna schimperi (Figure 5) is a spreading shrub or small tree which is quite common in East Africa at altitudes between 1300 and 2400 m. In central Ethiopia, it is known as "Chocho" (Amh) and is used in the treatment of inflammation and secondary infection associated with superficial wounds [99].

Figure 6. *P. schimperi* leaves (Ambo, APPRC garden Ethiopia)

The isolated secondary metabolites from this genus have been reported to display interesting biological activities including antioxidant, antibacterial, anti-inflammatory, cytotoxic and hepatoprotective [99-101].

In the chemical investigation of this genus, isolation and characterization of antibacterial diterpene compounds (**87**-**93**), different flavanones (**94**, **95**), and flavonols (**96**-**102**) were reported [102]. In another work, the antibacterial and antileishmanial activity of these labdane diterpenes were reported isolated from the genus *Premna* [103, 104].



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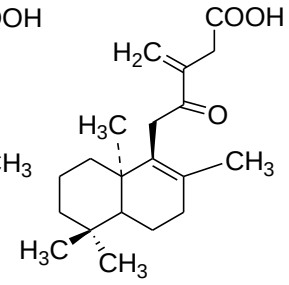

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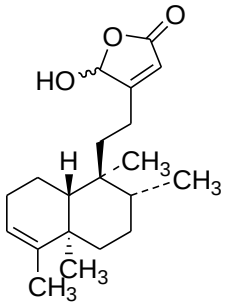

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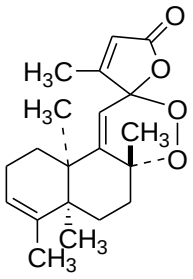

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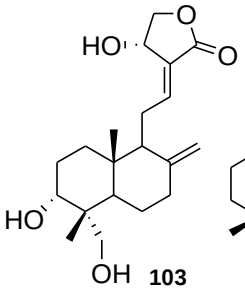

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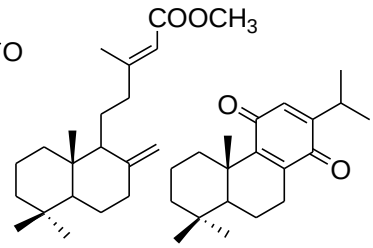

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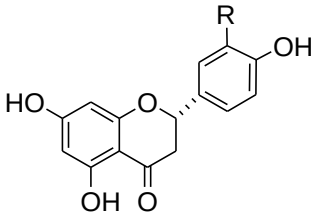
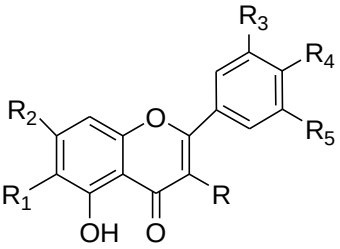


106. R = H

107. R = OH



Compound	R	R_1	R_2	R_3	R4	R5
108	OH	OMe	OMe	OMe	OMe	OH
109	OH	OMe	OH	OMe	OMe	OH
110	H	H	OH	OH	OH	H
111	OH	H	OH	OH	OH	H
112	OH	H	OH	H	OMe	H
113	OMe	H	OMe	OMe	OH	H
114	OMe	OMe	OMe	OH	OH	H

1.3. Statement and Scope of the Problem

The rapid spread of weeds like Parthenium in Ethiopia is becoming a risk to the expansion and sustainable production of many crops [105]. Control of weeds including Parthenium is therefore crucial to increase productivity and maintain the integrity of both agro-biodiversity and ecosystem complex of the country. Different controlling mechanisms of Parthenium weed were attempted worldwide. The controlling strategies have advantages and disadvantages. Hand weeding mostly used by small-scale farmers is more difficult due to the allergic effects of parthenium on the human body. Mechanical and chemical control strategies, on the other hand, are rather costly in terms of machinery, labor and time requirement. Furthermore, poor farmers of Ethiopia cannot afford to purchase herbicides. Also the use of herbicides is unsafe in terms of health and environmental considerations. Therefore, other options must be explored for effective, economic and ecological management of parthenium. One such option is the use of allelopathic plants that suppress germination and growth of the weed.

Allelopathy plays an important role in regulating plant diversity [106]. Phytotoxic and allelopathic compounds can be used as natural herbicides and they are less disruptive of the global ecosystem than synthetic agrochemicals [107]. Several reports address the importance of the allelopathic effect of various plants on parthenium weed germination and seedling growth. Aqueous extracts of *Azadirachta indica*, *Melia azadarach*, *Mangifera indica* [43] *, Albiza gummifera* and *Sesbania sasban* significantly reduced the germination and early seedling growth of *Parthenium hysterophorus* under laboratory and greenhouse conditions. In Ethiopia, there is no prior report on the search of phytotoxic compounds from natural sources.

Therefore, the main aims of this project are to search for biologically active compounds from some selected plant materials for the control of *Parthenium hysterophorus* weed and to characterize and elucidate the structures of these compounds.

2. Results and Discussion

2.1. Bio-assay results (*in vitro*)

On the basis of biological control of *P. hysterophorus* weed, fifty different plants (Table 2) were collected from different parts of Ethiopia and screened for their herbicidal activities on the target weed. The selection of five of the plants (*E. globulus, A. indica, M. indica, W. somnifera, M. azedarach*) was based on their previous phytotoxic information against the target weed and the remaining was based on our random searching of phytotoxic plants that could suppress the seed germination of the weed.

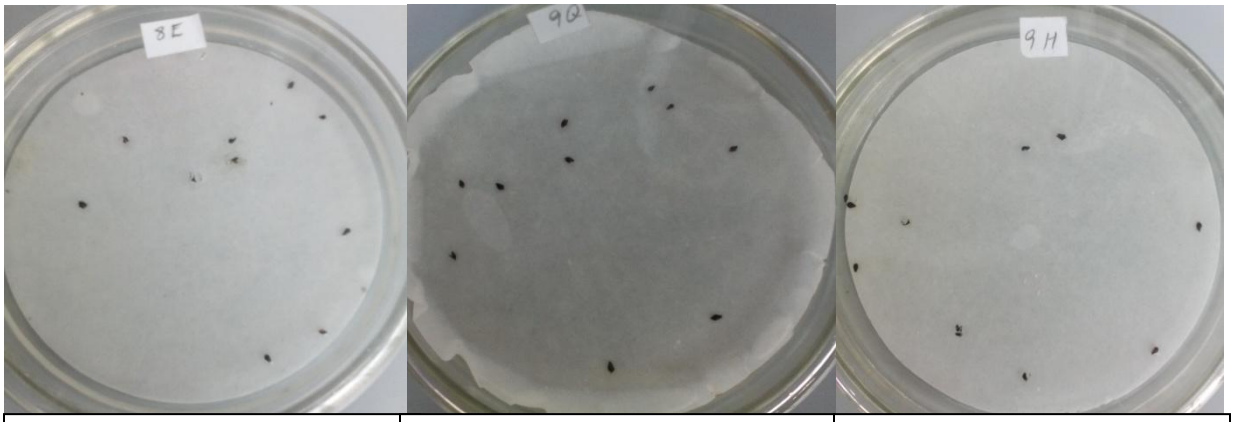
The *in vitro* seed germination inhibition response indicated that 20% of the EtOH extracts of the 50 plants exhibited phytotoxic activity against *Parthenium hysterophorus* weed at 1 mg/mL. Aerial parts of *Achyranthes aspera,* root of *Echinops kebericho,* leaves of *Solanecio gigas,* bark of *Warburgia ugandensis,* aerial parts of *Zehneria scabra,* leaves of *Eucalyptus globulus,* leaves of *Premna schimperi,* aerial parts of *Galium simense*, leaves of *Rhamnus prinoides* and both fruit and leaves of *Azadirachta indica* showed 100% seed germination inhibition *in vitro* at the concentration of 1 mg/mL. Those plants which showed herbicidal activities at 1 mg/mL were further diluted to concentrations of 0.02, 0.05, 0.1 and 0.2 mg/mL and their phytotoxic activities were further tested [108]. At 0.02 mg/mL concentration, all the fifty plants extracts showed insignificant activity below 20% seed germination inhibition. But at concentrations of 0.05, 0.1, 0.2 mg/mL, the EtOH extracts of *A. aspera, E. kebericho, S. gigas, W. ugandensis, Z. escabra* and *E. globulus* inhibited the seed germination with 100% GI *in vitro* (Figure 7).

Table 1. Effects of 0.02, 0.05, 0.1, 0.2 and 1mg/mL ethanol extract of selected plants on *P. hysterophorus* seed germination

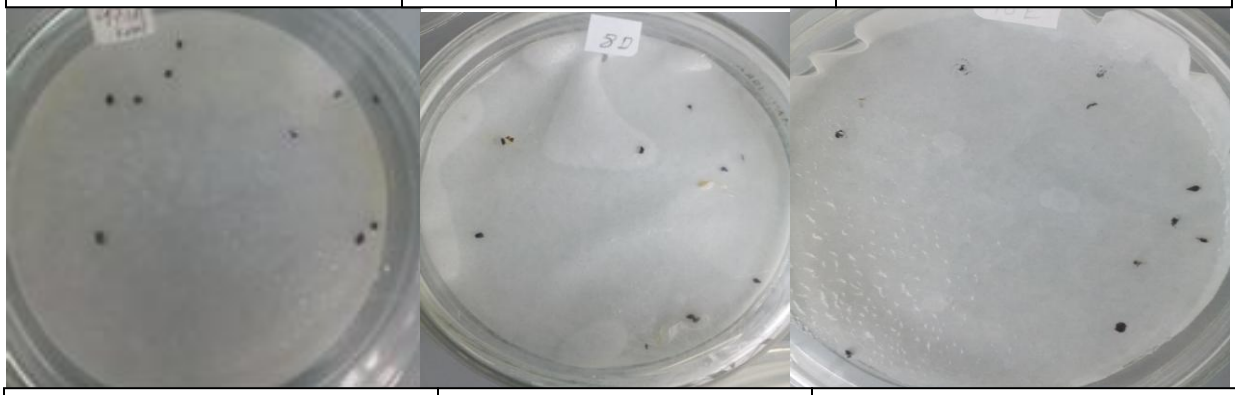
			Activity at different concentration (mg/mL)				
Species name	Local name	parts	0.02	0.05	0.1	0.2	100
A. aspera	Telenj	Ap	17	100	100	100	100
E. kebericho	Kebericho	Rt	27	100	100	100	100
R. prinoides	Gesho	Lf	9	85	85	94	94
S. gigas	Dengoreza	Lf	20	100	100	100	100
Z. scabra	Areg resa	Ap	13	100	100	100	100

W. ugandensis	Kenafa	Br	25	100	100	100	100
E. globulus	N. b/zaf	Lf	19	100	100	100	100
A. indica	Kinin zaf	Lf	25	100	100	100	100
G. simense	Ashkit	Ap	19	20	100	100	100
P.schimperi	chocho	Lf	13%	78	100	100	100

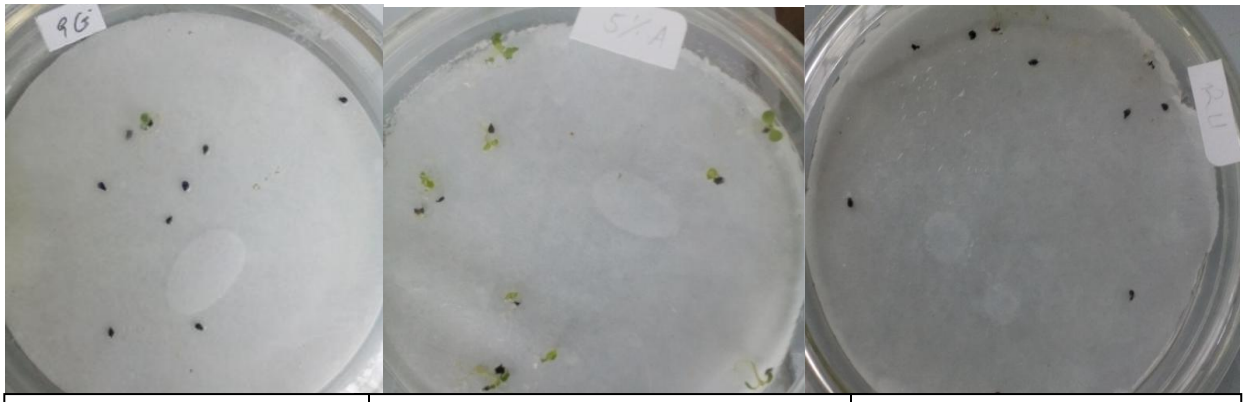
A. aspera, E. kebericho, *S. gigas, W. ugandensis, E. globules*, and *G. simense* displayed highest phytotoxic acitivity (100% GI) at 0.05 mg/mL. *P. schimperi* suppressed the parthenium seed germination by 78%. So, 0.05 mg/mL was taken as a minimum growth inhibition concentration in this work.



A. aspera (100% GI)	*E. kebericho* (100% GI)	*S. gigas* (100%GI)



W. ugandensis (100% GI)	*G. simense* (100% GI)	*E. globulus* (100% GI)

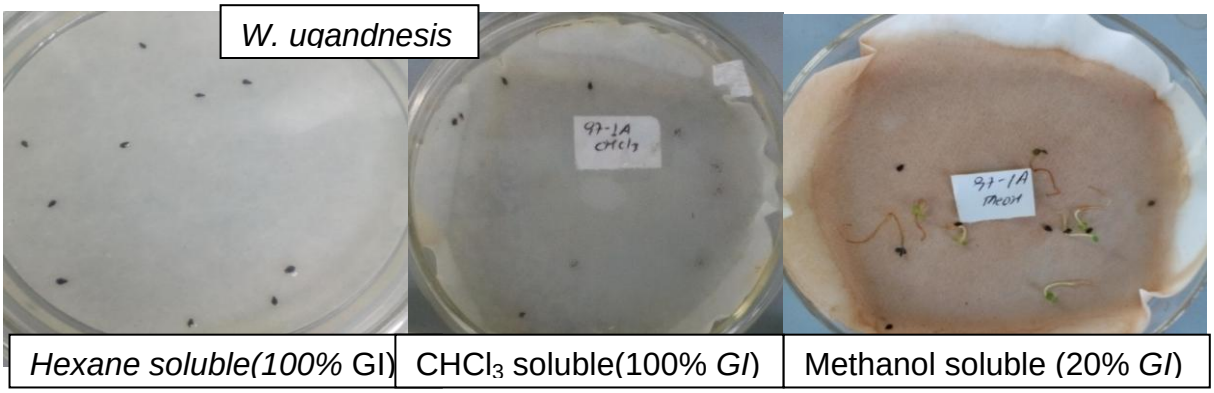


P. schimperi (78%GI)	5% acetone in water (-ve control)	Roundup® (+ve control)

Figure 7. Phytotoxicity results of selected plants at 0.05 mg/mL

Based on the above results, those plants that exhibited above 80% seed germination inhibition are considered as highly active, between 60 and 80% medium active plants. The remaining plants which exhibited below 60% seed germination inhibition are considered as poorly active.

The ethanol extracts of the above plants were partitioned with hexane, $CHCl_3$, and MeOH and tested for their phytotoxic activity against parthenium seed germination. The hexane and $CHCl_3$ soluble portions of the ethanol extracts of the above plants were found to be more active than methanol portion. The hexane and $CHCl_3$ soluble portions of ethanol extracts of bark of *W. ugandensis,* aerial parts of *A. aspera* and *G. simense, leaves of E. globules* and *S. gigas* inhibited parthenium seed germination with 100% at concentrations of 0.05 mg/mL. The MeOH soluble portion ethanol extracts of all plants showed relatively lower activity when it was compared with the nonpolar portion as it is shown in the figure below.



Hexane soluble(100% GI)	$CHCl_3$ soluble(100% *GI*)	Methanol soluble (20% *GI*)

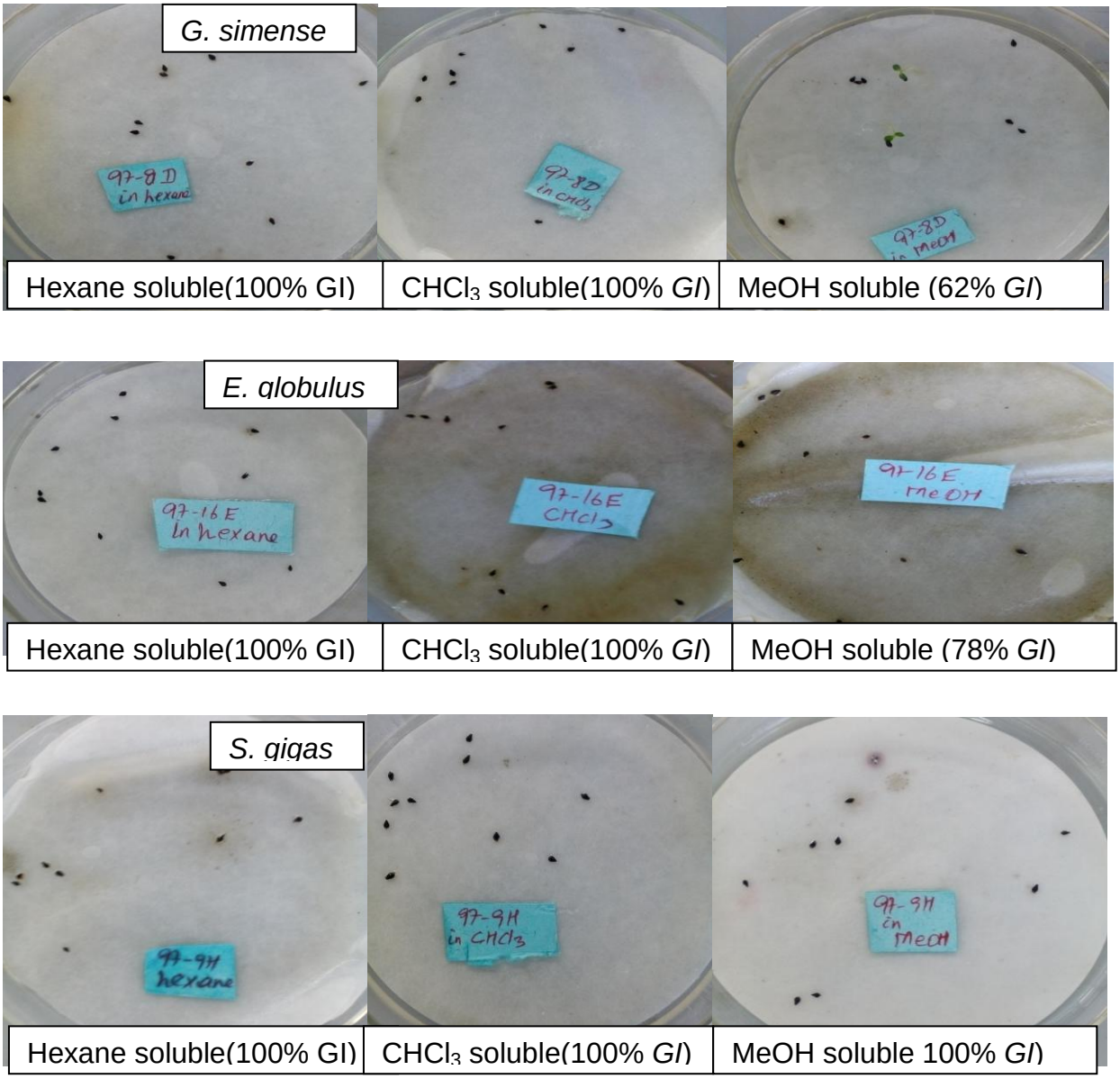
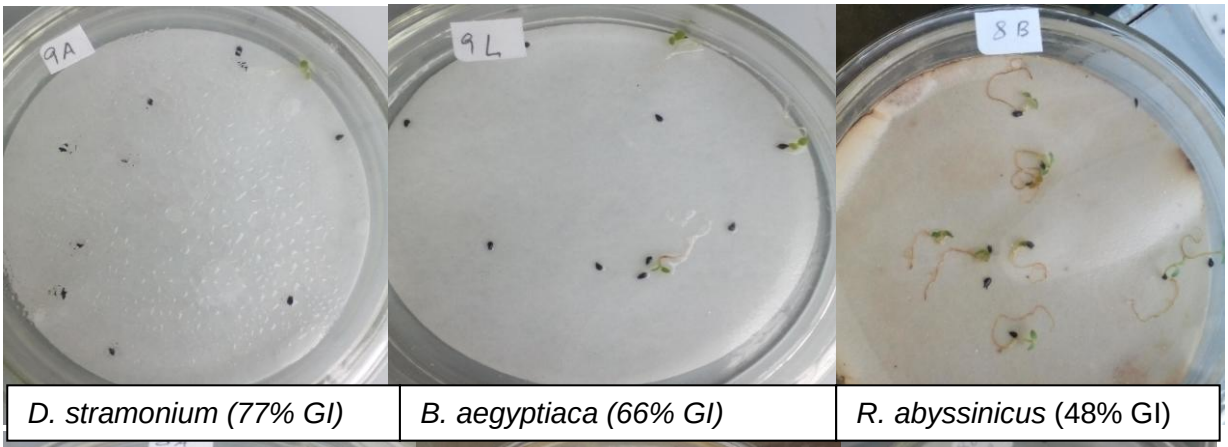


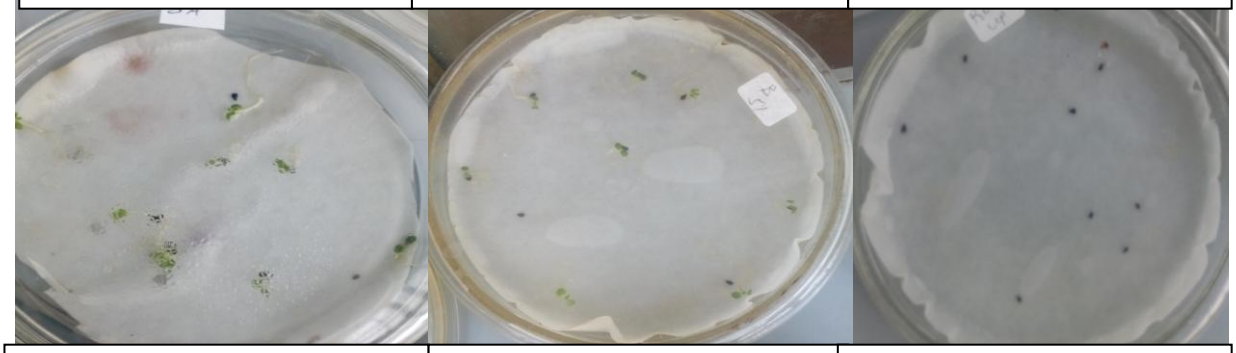
Figure 8. Phytotoxic effects of hexane, $CHCl_3$ and MeOH soluble portions of ethanol extracts of W. ugandensis, *G. simense, E. globules* and *S. gigas.*

Artemisia annua inhibited parthenium seed germination with 93% at 0.05 mg/mL concentration *in vitro*. This may be due to artemisinin which is previously reported to be active. But the level of herbicidal activity was due to the concentration of artemisinin in soil [15]. The phytotoxicity of ethanol extracts leaves of *Premna schimperi* was studied on *P. hysterophorus* seed germination and showed 78% GI *in vitro* at concentrations of 0.05 mg/mL. *Galium simense* and *Rhamnus prinoides* also exhibited an activity of 85% growth inhibition. An equivalent experiment was done with 5% acetone in water to study the effect of the solvent as a negative control and showed no effect on seed germination.

Commercial chemical roundup ®, which was used as a positive control, inhibited the seed germination with 100% GI at the same concentration of botanicals.



D. stramonium (77% GI)	*B. aegyptiaca (66% GI)*	*R. abyssinicus* (48% GI)



M. indica (18% GI)	*A. abyssinicus (26% GI)*	Round up *(100% GI)*

Figure 9. Some medium and inactive plants against *P. hysterophorus* weed *in vitro*

The presence of germination altering secondary metabolites was confirmed by counting the number of germinated seeds on the Petri dish after 15 days. Those plants which showed good phytotoxic activity were identified and their percentage germination inhibition were calculated using the standard formula (1-Gt/Gc) x100% [109]. The percentage growth inhibition of each plant is depicted in the table below.

Table 2. Phytotoxicity of ethanol extracts of plants at 5%

Species name	Local name	parts	T1	T2	T3	mean	STDV	%GI
Achyranthes aspera	Telenj	Ap	0	0	0	0	0	100
Echinops kebericho	Kebericho	Rt	0	0	0	0	0	100
Solanecio gigas	Dengoreza	Lf	0	0	0	0	0	100
Zehneria scabra	Areg resa	Ap	0	0	0	0	0	100
Warburgia ugandensis	Kenafa	Br	0	0	0	0	0	100
Eucalyptus globulus	Nech barzaf	Lf	0	0	0	0	0	100
Azadirachta indica	Kinin zaf	Lf	3	3	4	3.3	0.57	63
Artemisia annua	China chiqugn	Ap	0	0	2	0.6	1.15	93
Croton macrostachyus	Bisana	Lf	0	0	0	0	0	100
Galium simense	Ashkit	Ap	0	3	1	1.3	1.52	85
Rhamnus prinoides	Gesho	Lf	3	0	1	1.3	1.52	85
Premna schimperi	Chocho	Lf	3	1	2	2	1	78
Datura stramonium	Atse faris	Lf	4	1	1	2	1.73	78
Euclea divinorum	Dedeho	Lf	0	4	3	2.3	2.08	74
Ricinus communis	Gulo	Lf	0	4	3	2.3	2.08	74
Verbascum sinaiticum	Yahya joro	Lf	1	2	3	2	1	78
Verbascum sinaiticum	yahya joro	Fr	4	3	2	3	1	67
Jatropha curcas	Jatropha	Lf	2	2	1	1.67	0.57	81
Guizotia scabra	Gmie/mechi	Lf	0	3	6	3	3	67
Piper capense	Temiz	Fr	3	3	1	2.3	1.1	74
Osyris quadripartite	Qeret	Oil	4	3	4	3.6	0.57	59
Balanites aegyptiaca	Bedeno	Ap	4	3	4	3.6	0.57	59
Olinia rochetiana	Dalecho	Fr	2	3	6	3.6	2.01	59
Millettia ferruginea	Berbera	Ap	6	0	6	4	3.4	55
Cynoglossum coeruleum	Chigogot	Fr	3	4	5	4	1	56
Kniphofia foliosa	Ashenda	A	3	4	5	4	1	56
Olea xeuropea	Weyra	Lf	6	6	3	5	1.7	44
Matricaria recutita	Chamomile	Ap	4	6	5	5	1	44
Thymus schimperi	Tosign	Rt	6	4	5	5	1	44
Urtica simensis	Sama	Lf	6	6	5	5.6	0.6	37

Salvia officinalis	Sage (EnG)	Ap	5	9	3	5.6	3	37
Dodonia angustifolia	Ketketa	Lf	6	2	8	5.3	3	41
Allophylus abyssinicus	Embis	Lf	4	7	9	6.7	2.5	26
Vernonia amygdolina	Grawa	Lf	1	0	1	0.7	0.6	93
Sesbania sesban	Shewshewie	Lf	8	8	3	6.3	2.9	30
Cucumis prophetarum	Yemdr embuay	Fr	7	4	7	6	1.7	33
Withania somnifera	Gezawa	Wd	5	8	8	7	1.7	22
Salvia schimperi	Deberq	Ap	5	5	2	4	1.7	55
Eucalyptus citriodora	Shito b/zaf	Lf	9	8	4	7	2.6	22
Euphorbia platyphyllos	Anterfa	Ap	8	8	5	7	1.7	22
Peucedanum mattirolii	Sire bzu	Pt	9	9	3	7	3.5	22
Lippia adoensis	Koseret	Ap	6	7	8	7	1	22
Rumex abyssinicus	Mekmeko	Lf	3	7	4	4.6	2.1	48
Mangifera indica	Aleko	Lf	9	6	7	7.3	1.5	18
Albizia gummifera	Sesa	Lf	7	8	8	7.7	0.6	15
Melia azedarach	China berry	Lf	9	8	9	8.7	0.6	3
Syzygium guineense	Doqma	Fr	9	9	8	8.7	0.6	3
Moringa stenopetala	Moringa	Oil	9	9	9	9	0	0
Piper capense	Timz	Lf	8	8	10	8.7	1.2	3
Maesa lanceolata	Abaliyeh	Fr	8	8	9	8.3	0.6	7
Maesa lanceolata	Abaliyeh	Lf	8	9	0	5.6	4.9	37
Roundup (R^+)	+ve control		0	0	0	0	0	100
5% acetone in water	-ve control		9	9	9	9	0	0

2.2. *In vivo* bioassay results of *in vitro* active plants

Ethanol extracts of the bark of *W. ugandensis*, leaves of *S. gigas,* the root of *E. kebericho*, aerial parts of *Z. escabra*, leaves of *E. globulus* and aerial parts of *A. aspera* showed 100% parthenium seed germination inhibition *in vitro* at concentration of 0.05 mg/mL. On the bases of the *in vitro* result, the EtOH extracts of these plants were further screened for their herbicidal activity *in vivo* under greenhouse conditions at 1mg/mL and showed highest activities above 80%. The $CHCl_3$ soluble parts of ethanol extracts of *W. ugandensis, E. kebericho, S. gigas,* and *A. aspera* were the most effective fractions to

suppress the growth of the 15 days old seedlings by 94%, 92%, 85%, and 76% respectively (Table 3). The ethanol extracts of the leaves of *E. globulus* significantly inhibited growth of the seedling (60%). But, the activity of similar extracts of *Galium simense, Z. scabra* and *R. prinoides* was found insignificant (below 20%) *in vivo* even though it's *in vitro* seed germination result was promising (100% GI).

Table 3. *In vivo* bioassay results of the most active plants

EtOH extract of	ADB of seedlings before treatment (mg)				ADB of seedling after treatment (mg)				Mass difference	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
A. aspera	81	60	69	70	600	710	682	664	594	76
W. ugandensis	81	60	69	70	200	180	280	220	150	94
E. kebericho	81	60	69	70	352	140	310	267	197.5	92
S. gigas	81	60	69	70	420	400	490	437	366.7	85
G.simense	81	60	69	70	2217	2010	1995	2074	2004	19
P. schimperi	81	60	69	70	1050	1065	1070	1062	992	59
Roundup®	81	60	69	70	141	120	129	130	60	97
5% acetone	81	60	69	70	2515	2500	2620	2545	2475	0

E. kebericho (92%)	*W.ugandensis* (94%)	*S.gigas* (85%)	*A.aspera* (76%)

G. simense GI =19%	5% acetone in water (-ve control)	Roundup® (+ve control)

Figure 10. *In vivo* results under greenhouse condition

2.3. Bioassay-guided isolation of compounds from active plants

Based on the *in vitro* and *in vivo* bioassay results, *Echinops kebericho, Warburgia ugandensis, Solanecio gigas*, *Achyranthes aspera,* and *Premna schimperi* were candidates of active plants to find phytotoxic compounds against *Parthenium hysterophorus* weed. The chemical investigation of *Solanecio gigas* was unsuccessful. The crude ethanol extract was applied over silica gel column chromatography and led to the isolation of two compounds. The first compound, which NMR spectral data depicted below, is highly unstable and it was totally decomposed before its 2-D NMR experiment was done. The second compound is a straight chain alcohol with an insignificant amount (5 mg). Enlarge, the TLC profile of the leaf ethanol extract of this plant didn't show significant spots in different spraying agents. Since the aim of this project is to find phytotoxic compounds that could be screened in the laboratory and in greenhouse against *Parthenium hysterophorus* at a given standard set, the isolated compounds should be structurally stable with a significant amount. So, the activity observed with the crude extracts of this plant may be due to synergetic effects or the presence of potentially active compounds with minimum amount.

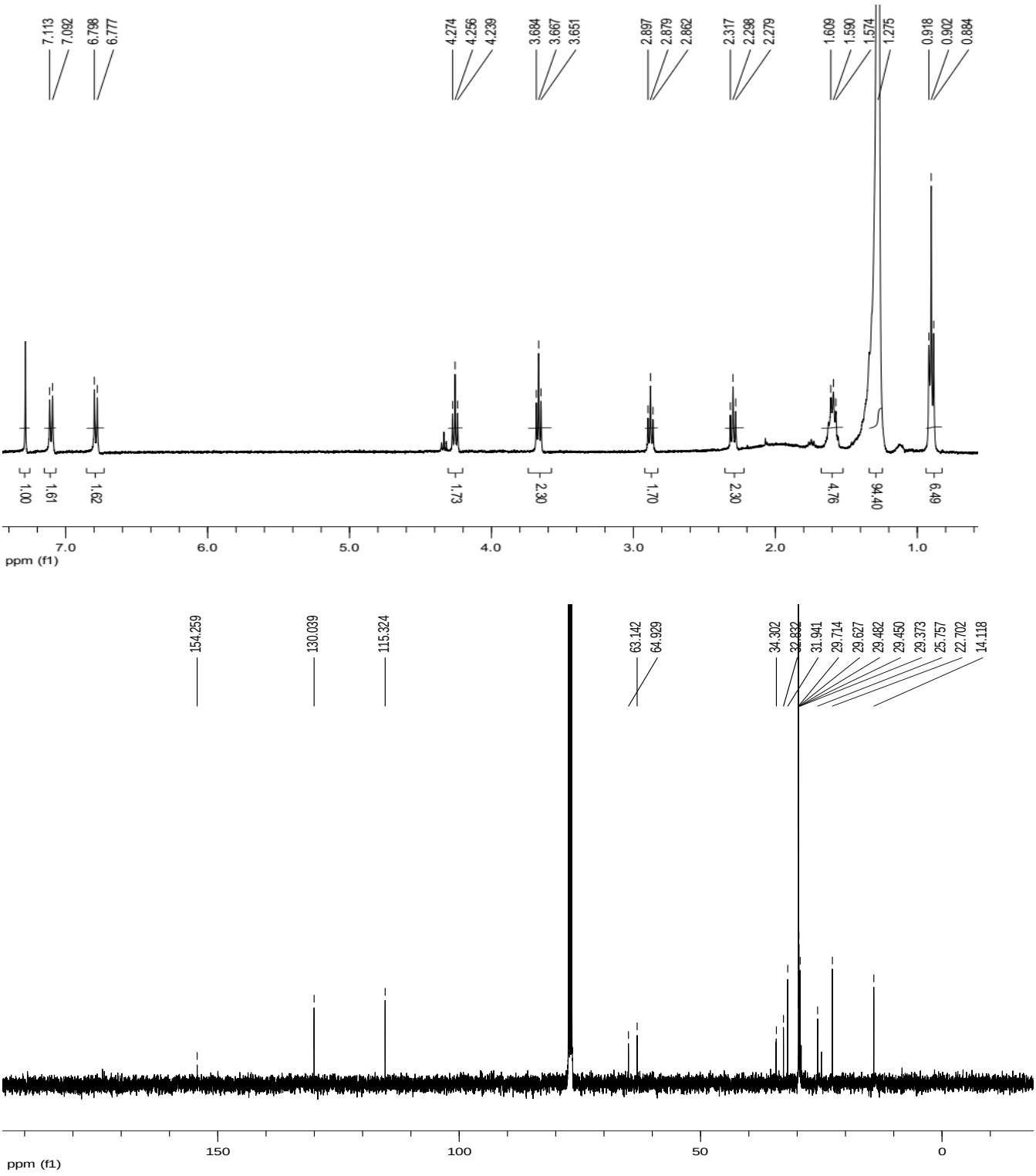


Figure 11. ^{1}H and ^{13}C-NMR spectral data of unknown compound isolated from *S. gigas*

2.3.1. Isolation of active compounds from the root of *E. kebericho*

The hexane, chloroform and methanol soluble portions of the EtOH extract of the root of *Echinops kebericho* were assayed for its phytotoxic activity against *Parthenium hysterophorus* weed. The $CHCl_3$ extract displayed better activity and hence subjected to further fractionation over silica gel column chromatography which led to the isolation of compound **28**. The characterization of this compound was done using various spectroscopic methods as briefly described below.

2.3.2. Characterization of compound **28**

Compound **28** was isolated as a white crystal (480 mg, 16%) from CC of $CHCl_3$ soluble portion of the ethanol extracts of *E. kebericho* root using hexane as eluent. The compound melts at 55-56^{o}C (lit. 60^{o}C) [110]. The TLC profile developed using hexane:EtOAc (4:1) as a mobile phase and vanillin as a spraying agent showed a blue spot (Rf 0.5).

The ^{1}H-NMR spectrum (Appendix 1) of compound **28** showed signals at *δ* 3.90 (1H, *t, J* = 9.2 Hz) assigned for an oxygenated methine proton. The multiplet signal observed at *δ* 2.85 was interpreted for three different methine protons. The presence of three exocyclic double bonds were confirmed by the signals appeared at *δ* 6.12 (1H, *d*) and 5.43 (1H, *d*), *δ* 5.19 (1H, *s*) and *δ* 4.99 (1H, *s*) and *δ* 4.83 (1H, *s*) and *δ* 4.74 (1H, *s*). The methylene protons appeared at *δ* 6.12 and 5.43 (each 1H) are coupled each other. But, the remaining exocyclic protons are observed as each as a singlet indicating they are not coupled each other.

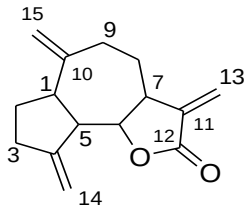
The ^{13}C-NMR spectrum of compound **28** together with DEPT 135 (Appendix 1) showed the presence of seven methylene (at *δ* 30.2, 30.9, 32.6, 36.3, 109.4, 112.5 and 120.1), four methine (at *δ* 45.0, 47.5, 51.9 and 85.3) and four quaternary (at *δ* 151.3, 149.2, 139.6 and 170.1) carbon signals. The ^{1}H and ^{13}C-NMR data of compound **28** were compared with the literature values of dehydrocostus lactone (DHCL) and found in a close agreement (Table 4) [60, 111].

Table 4. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **28** with literature values (400 MHz), $CDCl_3$) of DHCL

Experimental data of compound **28**			Literature report of DHCL [111, 112]	
	^{13}C-NMR ($CDCl_3$)	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	47.5	2.85 (1H, *m*)	47.5	2.9 (1H, *m*)
2	32.6	1.87 (2H, *m*)	32.5	1.9 (2H, *m*)
3	30.2	2.47 (2H, *m*)	30.2	2.5 (2H, *m*)
4	151.3		150.9	
5	51.9	2.85 (1H, *m*)	51.9	2.8 (1H, *m*)
6	85.3	3.90 (1H, *t,* 9.2)	85.1	3.9 (1H, *t, 9.3*)
7	45.0	2.85 (1H, *m*)	45.0	2.9 (1H, *m*)
8	30.9	2.20 (1H, *m*)	30.9	2.24 (1H, *m*)
		1.37 (1H, *m*)		1.46 (1H, *m*)
9	36.3	2.44 (1H, *m*)	36.2	2.52 (1H, *m*)
		2.10 (1H, *m*)		2.27 (1H, *m*)
10	149.2		148.8	
11	139.6		139.5	
12	170.1		170.0	
13	120.1	6.12 (1H, *d,* 3.6)	119.9	6.21 (1H, *d,* 3.4)
		5.43 (1H, *d*, 2.8)		5.49 (1H, *d,* 2.9)
14	112.5	5.19 (1H, *s*)	112.4	5.27 (1H, *d,* 2.2)
		4.99 (1H, *s*)		5.07 (1H, *d,* 2.2)
15	109.5	4.83 (1H, *s*) & 4.74 (1H, *s*)	109.4	4.90 (1H, *s*), 4.82 (1H,*s*)

Chemical shifts are reported in parts per million ($CDCI_3$,), *J* values are in Hertz.

Comparison of the NMR data of compound **28** with literature reported for dehydrocostus lactone agreed closely. So, compound **28** is proposed to be dehydrocostus lactone which was previously reported from *E. kebricho* [113] and exhibited different biological activities: such as, antibacterial, antifungal, insecticidal, antidiarrheal and ex-vivo spasmolytic activities.



28

2.3.3. Germination inhibition of dehydrocostus lactone (**28**)

The seed germination inhibition effects of dehydrocostus lactone, tested with triplicate experiment, showed 100% GI at 0.05 mg/mL concentration (Table 5).

Table 5. *In vitro* bioassay result of dehydrocostus lactone

compound	source	number of germinated seeds					
		T1	T2	T3	mean	STDV	%GI
Dehydrocostus	*E.k*	0	0	0	0	0	100
Roundup®		0	0	0	0	0	100
5% acetone		9	8	8	8.3	0.57	0.79

Compound **28** (DHCL) GI =92%	*5% acetone in water (- ve contro*l), GI= 0%	*Roundup® (+ve control)* GI = 97%

Figure 12. *In vivo* herbicidal activity of DHCL against parthenium weed

In order to determine the shelf life and time variation effects of dehydrocostus lactone, the screening test was done two times in two months time interval and showed above 90% phytotoxic activity (85% at 4000 ppm) [112] against *P. hysterophorus* weed.

The herbicidal potential of dehydrocostus lactone was also studied *in vivo* under greenhouse condition and displayed very high phytotoxic activity (92%) against Parthenium weed seedling growth as the standard positive control roundup® (Figure 11). The growth of 15 days old parthenium weed seedlings was totally inhibited at 1 mg/mL concentration. The herbicidal property of this compound against two kinds of grasses and two broadleaf plants was previously reported [60]. When DHCL was applied at 4000 ppm, greater than 85% necrotic injury was obtained from large crabgrass, maize, and soybean, whereas only about 40% necrotic injury appeared in black nightshade, indicating that DHCL has no gross morphological selectivity [60].

Based on this, we conclude that dehydrococtus lactone is not selective to *Parthenium hysterophorus* and can damage other weeds at lower oncentation than 1 mg/mL by yellowing the leaves after two days of foliar contact.

2.3.4. Bioassay results of ethanol extracts of *Warburgia ugandensis*

Ethanol extracts of bark of *W. ugandensis* inhibited parthenium seed germination by 100% at 1 mg/mL. This stock solution was then serially diluted to 0.02, 0.05, 0.1and 0.2 mg/mL for bioassay test. The result showed that the ethanol extract of *W. ugandensis bark* suppressed the seed germination by 100% at 0.05, 0.1 and 0.2 mg/mL. Whereas, only 25% seed germination inhibition was observed at concentration of 0.02 mg/mL. The crude ethanol extract of bark of *W. ugandensis* was partitioned with hexane, $CHCl_3$ and MeOH and screened for phytotoxicity effect. The result disclosed that the nonpolar soluble portions (hexane and $CHCl_3$) showed 100% seed germination inhibition (*in vitro*) and above 87% *in vivo*. But, the polar MeOH soluble part showed lower than 20% seed germination and seedling growth inhibitions both *in vitro* and *in vivo*.

2.3.5. Isolation of compounds from stem bark of *W. ugandensis*

The bark of *W. ugandensis* was extracted with EtOH to afford 15 g (10%) red like jelly material. The extract of the bark of *W. ugandensis* was analyzed with TLC (Figure 13) using hexane:EtOAc (1:1) as a mobile phase and showed more than ten spots.

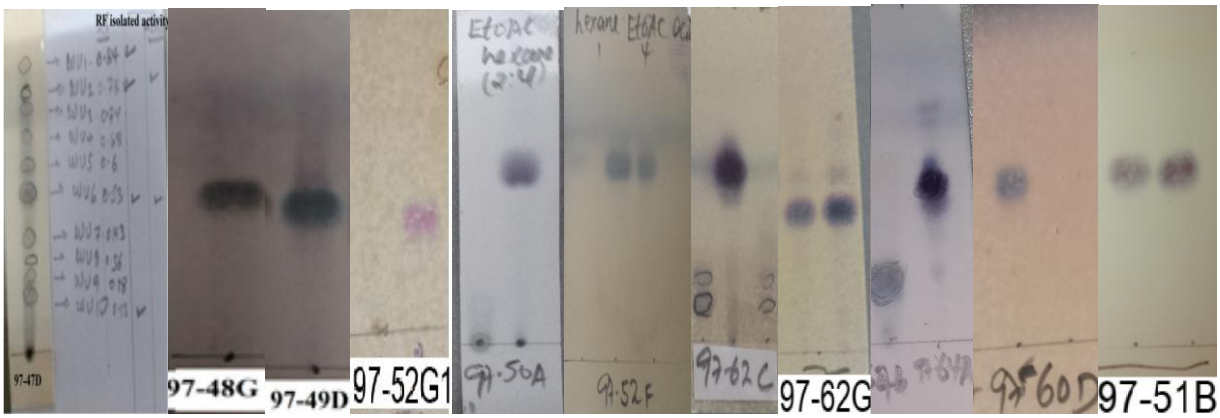


Figure 13. TLC of crude extracts and pure compounds isolated from of *W. ugandensis* bark

The EtOH extract was partitioned with EtOAc which furnished 10 g (6.6%). The bioassay-directed chromatographic fractionation of EtOAc soluble portion of EtOH extract of the bark of *W. ugandensis* resulted in the isolation of twelve compounds identified using various spectroscopic methods such as muzigadial (**48**), cinnamolide-3*β*-acetate (**64**), *β*-sitosterol (**86**), ugandensidial (**60**), 11α-hydroxymuzigadiolide (**66**), albicanyl acetate

(**115),** polygodial (**49**), 9-deoxymuzigadial (**116**), caseamemin (**117**)**,** 1-heptacosanol (**118**), Hentriacontane (**119**) and compound **120**. The structural elucidations of these compounds were carried out on the basis of physical and spectroscopic analysis and comparing the data with related compounds reported in the literature and the result is presented below.

2.3.6. Characterization of compounds isolated from *W. ugandensis*

Compound 48

Compound **48** was isolated as a white crystal (250 mg) from column chromatographic fractionation of the EtOAc soluble portion of the EtOH extract of the bark of *W. ugandensis* with hexane:EtOAc (4:1) as eluent. The compound melts at 120-122^{o}C (lit. l22-124°C) [73]. The TLC profile developed using hexane:$CHCl_3$:EtOAc (1:1:1) as a mobile phase was visualized as single greenish spot (Rf = 0.42) after spraying with vanillin in H_2SO_4 and heating with a hot air gun (Figure 13).

The UV-Vis spectrum (in EtOH) showed absorption maxima at 223.6 nm indicates the presence of *α, β*-unsaturated carbonyl group. The IR spectrum showed absorption band at 3462 cm^{-1} is an indicator of the presence of OH functional group. The peak appeared at 2957 cm^{-1} is due to C-H stretching. Two sharp peaks appeared at 1720 and 1663 cm^{-1} are due to α, *β*-unsaturated carbonyl group. The peaks observed at 1638 and 900 cm^{-1} are the characteristic peaks of an exocyclic methylene group. This data is in the fingerprint region with literature report of muzigadial [73].

^{1}H-NMR spectrum (Appendix 2) of compound **48** displayed signals at *δ* 9.64 (1H, *s*) and *δ* 9.44 (1H, *s*) which are due to two aldehydic protons. A proton signal at *δ* 7.24 (1H, *t*) is assigned to a *β*- proton of *α, β* unsaturated group. Two signals observed at *δ* 4.93 (1H, *s*) and *δ* 4.76 (1H, *s*) are assigned to methylene protons of the exocyclic double bond. The presence of upfield signals at *δ* 0.88 (3H, *s*) and at *δ* 1.06 (3H, *d*) is evident for the presence of two terminal methyl group in the compound.

^{13}C-NMR together with the DEPT-135 spectrum of compound **48** displayed 15 well-resolved carbon signals. From these, four are due to quaternary, two methyls, four methylene, and three methines. Downfield signals observed at *δ* 201.3 and *δ* 192.7 in the

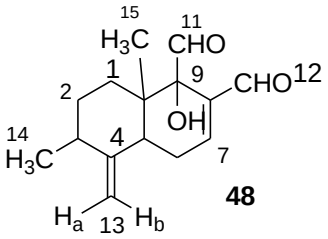
^{13}C-NMR spectrum together with the help of ^{1}H-NMR spectrum suggested the presence of two aldehydic carbons in the molecule. Other signals observed in the ^{13}C-NMR spectrum at *δ* 139.8 and *δ* 155.8 were assigned to *α* and *β* carbons of *α, β* unsaturated group respectively. The olefinic carbon signals appeared at *δ* 106.1 and *δ* 151.1 is evident for the presence of exocyclic double bond in the compound. The ^{1}H and ^{13}C-NMR data were in close agreement with the literature report of muzigadial [114] isolated from the same plant (Table 6).

Table 6. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **48** literature values of Muzigadial (400 MHz), $CDCl_3$)

Experimental result of compound **48**			Literature report of muzigadial [114]	
	^{13}C & ^{1}H-NMR ($CDCl_3$)		^{13}C & ^{1}H-NMR ($CDCl_3$)	
1	31.7	1.03 (1H, m)	31.0	1.04 (1H, ddd, 13, 4, 4)
		1.98 (1H, *td,* 13, 4)		1.99 (1H, *ddd,* 13,13, 4)
2	31.8	1.13 (1H, *td,*13, 4)	31.9	1.12 (1H, *dddd*, 13,13,13, 4)
		1.69 (1H, *m*)		1.70 (1H,*dddd,* 13, 4, 4, 4)
3	38.3	1.71 (1H, m)	38.3	1.90 (1H, *m*)
4	151.6		151.6	
5	40.2	2.51 (1H, m)	40.4	2.46 (1H, *m*)
6	27.6	2.61 (2H, *m*)	27.7	2.50 (2H, *m*)
7	155.8	7.23 (1H, *t,* 4)	155.2	7.24 (1H, *dd,* 5, 3)
8	139.8		140.1	
9	77.6	4.07 (1H, *d,* 1.2)	77.7	4.05 (1H, *br s*)
10	42.3		42.5	
11	201.3	9.63 (1H, *s*)	200.9	9.62 (1H, *br s*)
12	192.7	9.44 (1H, *s*)	192.4	9.41 (1H, *s*)
13	106.1	4.93 (1H, *s*)	105.9	4.93 (1H, *dd,* 3, 3)
		4.77 (1H, *s*)		4.75 (1H, *dd,* 3, 3)
14	18.4	1.07 (3H, *d,* 6.4)	18.5	1.06 (3H, *d,* 6)
15	15.1	0.86 (3H, s)	15.5	0.85 (3H,*s*)

Chemical shifts are reported in parts per million ($CDCl_3$), ***J*** values are in Hertz.

Based on the interpretation of the physical and spectroscopic data and literature comparison, compound **48** was proposed to be muzigadial. Its structure is depicted below. The herbicidal activity of compound **48** was carried out both *in vitro* and *in vivo* and the result showed strong activity (95% GI). Muzigadial was previously isolated from this plant by different scholars and showed antifeedant activity against the monophagous African army worm. In addition to this, the compound possessed potent molluscicidal and antibiotic properties [73].



48

Compound 64

Compound **64** (50 mg) was obtained as a white powder from fraction 7 and 8 of hexane:EtOAc (4:1) solvent system. The melting point of the compound is 141-142^{o}C (lit. 147-149^{o}C) [64]. The TLC profile (Figure 13) developed with hexane:EtOAc (3:2) solvent system as a mobile phase showed a bluish spot (Rf 0.42) after visualized with vanillin in H_2SO_4.

The UV-Vis (in ethanol) spectral analysis showed absorption maxima at 224 nm which is due to the π-π* transition of electrons in α*, β*-unsaturated lactone group. The IR spectrum displayed the presence of *α, β*-unsaturated lactone at 1763 and 1680 cm^{-1}. This IR spectrum of compound **64** is in the fingureprint region with the literature values reported for α, β-unsaturated lactone (1760 and 1683) of Cinnamolide-3*β*-acetate [64]. The presence of the acetate group is confirmed by the IR absorption at 1730 cm^{-1}.

The ^{1}H-NMR spectrum (Appendix 3) of compound and an acetate group (1731 cm^{-1}) **64** displayed signals at *δ* 6.90 (1H, *dd*, *J* = 3.2, 3.6 Hz, H-7) for olefinic proton. The signal observed at *δ* 4.55 (1H, *dd*, *J* = 11.6 Hz, 4.0, H=3) is due to the oxygenated proton of C-3 that bears the acetylated group. The ^{1}H-NMR spectrum showed signals at *δ* 4.06 (1H, *t*, *J* = 5.95 Hz) and *δ* 4.41 (1H, *t*, *J* = 9.2 Hz) for the oxygenated protons of the lactone ring (C-11). The proton signals appeared at *δ* 0.84 (3H, *s*, H-15), *δ* 0.94 (3H, *s*, H-13), *δ* 1.00

(3H, *s,* H-14) and *δ* 2.07 (3H, *s,* acetate group) were assigned for terminal methyl protons. A doublet of doublet signal integrating for one proton at *δ* 1.47 (1H, *dd*, *J* = 11, 5.4) was assigned to methine proton (H-5).

The ^{13}C-NMR spectrum (Appendix 3) and DEPT-135 indicated the presence of well resolved resonances of seventeen carbons. These are four methyls, four methylene, four methines and five quaternary carbons. The two extreme downfield signals at *δ* 170.8 and *δ* 169.8 were assigned to carbonyl carbons of the acetate and the lactone group respectively. The signal observed in the ^{13}C-NMR spectrum at *δ* 135.88 and *δ* 127.1 were assigned to olefinic carbons of *α, β* unsaturated group of the lactone. Two oxygenated carbon signals appeared at *δ* 80.1 and *δ* 66.9 were interpreted to a carbon bearing an acetate functional group (C-3) and a methylene carbon of the lactone ring (C-11) respectively. The ^{1}H and ^{13}C NMR data of compound **64** were compared with literature reported for cinnamolide-3β-acetate [70] (Table 7) and found to be in good agreement.

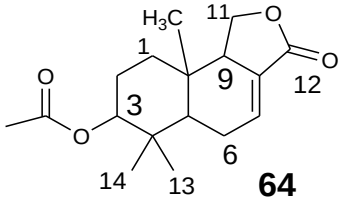
Table 7. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **64** literature values of Cinnamolide-3*β*-acetate (500 MHz), $CDCl_3$)

NMR spectral data of compound **64**			Literature report of Cinnamolide-3*β*-acetate	
	^{13}C-NMR	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR	^{1}H-NMR [70]
1	36.8	1.42 (1H, m)	36.9	1.42 (1H, *ddd*, 13.5, 4)
		1.64 (1H, *m*)		1.64 (1H, *dt,* 13.5, 3.5)
2	23.5	1.69 (1H, *m*)	23.5	1.68 (1H, *ddd*, 12, 3.5)
		1.74 (1H, *ddd*, 8, 4.4)		1.74 (1H, *ddd*, 13, 4)
3	80.1	4.55 (1H, *dd,* 11, 4.4)	80.2	4.55 (1H, *dd*, 11.5, 4.5)
4	37.6		37.7	
5	49.2	1.47 (1H, *dd,* 11, 5.4)	49.4	1.48 (1H, *dd,* 10.5, 4.5)
6	24.6	2.22 (1H, *m*)	24.7	2.22 (1H, *ddq,* 12, 3.5, 1.5)
		2.45 (1H, dq, 20, 4)		2.44 (1H, *dq*, 20, 5, 4)
7	135.8	6.90 (1H, *dd,* 3.2, 3.6)	135.7	6.89 (1H, *q*, 3.5)
8	127.1		127.2	
9	50.54	2.83 (1H, *m*)	50.6	2.82 (1H, *m*)
10	33.9		34.1	
11	66.9	4.05 (1H, *t*, 9) 4.41 (1H, *t*, 9)	66.9	4.05 (1H, *t*, 9, H-11β) 4.41 (1H, *t*, 9, H-11α)

12	169.8		169.7	
13	27.7	0.94 (3H, *s*)	27.6	0.94 (1H, s)
14	15.9	1.00 (3H, *s*)	15.9	1.00 (3H, *s*)
15	13.4	0.84 (3H, *s*)	13.5	0.85 (3H,*s*)
CH_3-CO-	21.2	2.09 (3H, *s*)	21.2	2.07 (3H, *s*)
CH_3-CO-	170.8		170.7	

Chemical shifts are reported in parts per million ($CDCl_3$), ***J*** values are in Hertz.

Based on the physical and spectroscopic interpretation of compound **64** and comparison of all necessary data with literature reported for cinnamolide-3*β*-acetate, compound **64** is proposed to be cinnamolide-3*β*-acetate.



64

This compound was previously isolated from the bark of *W. ugandensis* and exhibited antimycobacterial activity against *Mycobacterium aurum, M. fortuitum, M. phlei* and *M. smegmatis* [70]. In this study it is determined that compound **64** is a *Parthenium hysterophorus* weed growth suppressant by 91% GI.

Compound 60

Compound **60** was found in the form of a mixture with compound **64** as a white crystal. The two were separated into their pure form by using preparative thin layer chromatography (PTLC) using hexane:EtOAc (7:3) a mobile phase. Compound **60** (10 mg) was obtained as pure needle type crystal and it melts at 134-135^{o}C (lit.135-137^{o}C) [115]. The TLC profile (Figure 13) of compound **60** developed using hexane:EtOAc (4:1) solvent system as a mobile phase and vanillin in H_2SO_4 as a spraying agent confirms the presence of single red spot (Rf 0.48).

UV-Vis spectrum (in ethanol) of compound **60** showed absorption maxima at 223.4 nm indicating the presence of π-π* transition of electrons in α, *β*-unsaturated aldehyde functional group.

The IR spectrum displayed broad absorption peaks at 3453 cm^{-1} which confirms the presence of O-H stretching in the molecule. The intense absorption peak at 1736 is due to the acetate functional group in the molecule. The signal at 1680 and 910 cm^{-1} attributed to the *α, β*-unsaturated functional group in the molecule [73]. The peak at 2927 cm^{-1} was assigned to C-H stretching in the molecule. The band displayed at 1370 cm^{-1} is for geminal dimethyl stretching. The IR spectrum showed absorption at 1230 and 1024 cm^{-1} suggestive for the C-O stretching and bending.

^{1}H-NMR spectrum (Appendix 4) of compound **60** revealed the presence of two aldehydic proton signals at *δ* 9.50 (1H, *s*) and *δ* 9.78 (1H, *s*). A proton signal integrated for one proton appeared at *δ* 7.02 (1H, *d, J* = 4.8 Hz) is assigned to an olefinic proton. The ^{1}H-NMR spectrum showed triplet signal of a single proton at *δ* 5.91 (1H, *t*, *J* = 4.8 Hz) suggestive of the presence of an oxygenated proton. The presence of four singlet signals at *δ* 2.16 (3H, *s*), *δ* 1.35 (3H, *s*), *δ* 1.18 (3H, *s*) and *δ* 1.04 (3H, *s*) were assigned for four terminal methyl groups on quaternary corbons..

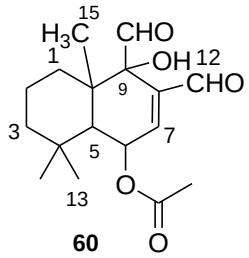
The ^{13}C-NMR spectrum of compound **60** demonstrated the presence of two aldehydic carbon signals at *δ* 201.2 and *δ* 193.1. The signal at *δ* 170.0 was assigned to an ester carbonyl carbon and the remaining downfield signals showed at *δ* 148.7 and *δ* 140.8 were accounted to *α, β* unsaturated olefinic carbons. The presence of oxygenated carbon at *δ* 77.3 was confirmed with the help of HMBC long-range correlation with protons of H-6, H-11, and H-12. Other oxygenated carbon signals appeared at *δ* 66.0 was assigned to the carbon that bears the acetate unit. The ^{1}H and ^{13}C-NMR spectrum of compound **60** was compared with the literature values of cinnamodial (ugandensidial) and found in a close agreement (Table 8) [115].

Table 8. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **60** literature values of cinnamodial ($CDCl_3$)

NMR spectral data of compound **60**			Literature report of cinnamodial [115]	
^{13}C-NMR of **60**		^{1}H-NMR of **60**	^{13}C-NMR	^{1}H-NMR of cinnamodial
1	31.7		31.9	
2	19.9		19.9	
3	43.9		44.2	
4	34.0		34.0	
5	44.9	2.05 (1H, *d*, 4.8)	45.2	2.05 (1H, *d*, 4.6)
6	66.0	5.91 (1H, *t*, 4.8)	66.2	5.87 (1H, *t*, 4.6)
7	148.7	7.02 (1H, *d,* 4.8)	148.5	7.04 (1H, *d*, 4.6)
8	140.8		141.3	
9	77.3	4.03 (1H, *br s,* OH)	77.54	4.01 (1H, *br s*)
10	41.6		41.7	
11	201.2	9.78 (1H, *s*)	201.0	9.75 (1H, *s*)
12	193.1	9.50 (1H, *s*)	192.9	9.47 (1H, *d,* 1.5)
13	32.5	1.34 (3H, s)	32.6	1.35 (3H, *s*)
14	21.4	1.17 (3H, s)	21.3	1.18 (1H, *s*)
15	17.6	1.01 (3H, s)	17.7	1.04 (3H, *s*)
CH3-CO-	24.7	2.14 (3H, *s*)	24.7	2.16 (3H,*s*)
CH3-CO-	170.0		170.0	

Chemical shifts are reported in parts per million ($CDCl_3$), ***J*** values are in Hertz.

Based on this spectroscopic data correlation, compound **60** is proposed to be cinnamodial (ugandensidial).



60

The previous study indicated that this compound was isolated from the bark of *Warburgia ugandensis* and exhibited a strong antifeedant activity [116] cytotoxic activity [115] . The presence of compound 60 in the bark of *Warburgia ugandensis* is significant for the phytotoxic acitivity of the plant.

Compound 86

Compound **86** was isolated as a white solid (27 mg) from the column chromatographic fractionation of the EtOAc soluble portion of the EtOH extract of bark of *W. ugandensis* with EtOAc:hexane (9:1) as eluent. It melts at 130-136^{o}C (lit. 134-136^{o}C) [117]. The TLC profile (Figure 13) developed using EtOAc:hexane (9:1) solvent system and vanillin as a spraying agent showed a purple single spot (Rf 0.62).

The UV-Vis spectrum (in ethanol) showed no absorption band from 600-200 nm wavelength region.

The most downfield signal in the ^{1}H-NMR spectrum (Appendix 5) at δ 5.36 is accounted to one olefinic proton. On the other hand the signal resonating at δ 3.53 is attributed to the presence of proton on a carbon bearing oxygen. as a multiplet for a proton corresponding to the proton connected to the C-3 hydroxyl group. The ^{1}H-NMR spectrum revealed the presence of 6 methyl groups of which three are methyl singlets at δ 0.82 (3H, *s*), 0.70 (3H, *s*), 1.03 (3H, *s*) and the other two are observed as methyl doublets at δ 0.86 (3H,*d*) and 0.93 (3H,*d*) and the remaing is methyl triplet at 0.84 (3H, *t*).

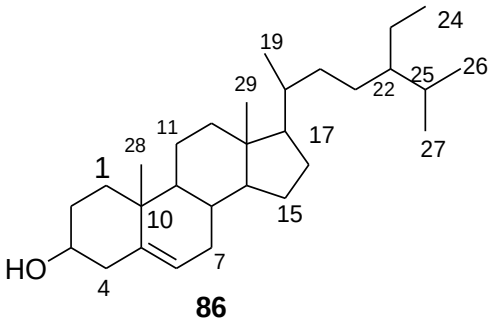
The ^{13}C-NMR spectrum (Appendix 5), showed downfield signals at δ 140.7 and 121.7 for olefinic carbons. In the ^{13}C-NMR, one methine oxygenated signal was observed at at δ 71.8. ^{1}H and ^{13}C-NMR data generated for compound **86** was consistant with the very well known β-sitosterol. The generated data and literature report are shown in Table 9 [117].

Table 9. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **86** and NMR data (600 MHz, $CDCl_3$) reported for *β*-sitosterol

	^{13}C & ^{1}H-NMR data of compound **86**		Lit. value		^{13}C & ^{1}H-NMR data of compound **86**		Lit. value [117]
1	37.2		37.5	16	28.2		28.5
2	31.6		31.9	17	56.0		56.3
3	71.8	3.53 (1H, *m*)	72.0	18	36.1		36.3
4	42.3		42.5	19	19.4	0.93 (3H,*d,* 6.8)	19.2
5	140.7		140.9	20	33.5		34.2
6	121.7	5.37 (1H, *t,* 2.8)	121.9	21	24.3		26.3
7	31.9		32.1	22	45.8		46.1
8	31.9		32.1	23	23.0		23.3
9	50.1		50.3	24	12.0	0.84 (3H, *t,* 7.2)	12.2
10	36.5		36.7	25	29.1		29.4
11	21.1		21.3	26	19.8	0.84 (3H, *d,* 6.8)	20.1
12	39.7		39.9	27	19.0	0.82 (3H, *d,* 6.8)	19.1
13	42.3		42.6	28	18.8	0.70 (3H, *s*)	19.0
14	56.7		56.9	29	11.8	1.03 (3H, *s*)	12.0
15	26.0		26.3				

Chemical shifts are reported in parts per million ($CDCl_3$), ***J*** values are in Hertz.

The data generated from the characterization of compound **86** agreed well with the literature report of *β*-sitosterol. Eventhough there is no former report for the isolated of *β*-sitosterol from stem bark of *W. ugandensis*, it was previously reported from other different plant sources and known for its anticancer, anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, spermicidal activities [118].



86

Compound 66

Compound **66** (25 mg) was isolated as a white amorphous solid after CC of the EtOAc soluble portion of the EtOH extract of bark of *W. ugandensis* with hexane:EtOAc as eluent. The compound melts at 181-182^{o}C (lit 182-183^{o}C) [85]. The TLC profile (Figure 13) of the compound developed using hexane:EtOAc (4:1) and two drops of formic acid as a mobile phase showed a dark bluish spot (Rf 0.52) after spraying with vanillin spraying agent.

The UV-Vis spectrum of the compound showed absorbance at 217 nm indicating the absence of conjugation in the structure. The IR spectrum exhibited bands at 3387 cm^{-1} for O-H stretching and 2961 and 2854 cm^{-1} for C-H stretching and bending respectively. Sharp peaks at 1704 and 1630 cm^{-1} indicates *α, β*-unsaturated lactone in the molecule [73]. From the IR spectrum the following indications were evident: bands at 3387 cm^{-1} for OH stretching, 2961 and 2854 cm^{-1} CH, 1704 and 1630 cm^{-1} for *α, β*-unsaturated lactone and 1171 cm^{-1} for C-O stretchings.

The ^{1}H-NMR spectra of compound **66** (Appendix 6) showed the presence of two terminal methyl groups at δ 0.77 (3H, *s*) and δ 1.08 (3H, *d*, J=6.8 Hz). The downfield signal observed at δ 6.90 (1H, *s*) was assigned for the anomeric proton (H-11). The other downfield signal in the ^{1}H-NMR spectrum appeared at 6.31 (H, *dd*, *J* = 0.8, 1.2 Hz) is due to the olefinic proton H-7. A broad spectrum of a single proton at δ 5.7 (1H, *br s*) was assigned to a proton on oxygen attached to C-11. The ^{1}H-NMR spectrum of compound **66** revealed the presence of two singlet signals at *δ* 4.80 (1H, *s*) and *δ* 4.90 (1H, *s*) supportive for terminal protons of the exocyclic double bond. A broad spectrum observed

at *δ* 3.07 (1H, *br s*) assigned for a proton of OH functional group. A signal appeared at a triplet of doublet at *δ* 2.36 (2H, *td*) was assigned for a proton of a methylene group (carbon 2).

The ^{13}C-NMR spectrum together with DEPT-135 revealed the presence of two methyls, four methylene, four methine and five quaternary carbon atoms. The downfield signal appeared at *δ* 166.4 was evident for the presence of a carbonyl group. The presence of two double bonds within the molecule was confirmed by the signals appeared at *δ* 138.3 and *δ* 130.9 for *α, β*-unsaturated carbons and at *δ* 152.6 and *δ* 104.9 for the exocyclic double bond. The presence of one anomeric carbon was evident due to the signals observed at *δ* 98.2. The ^{1}H and ^{13}C-NMR spectral data generated from the characterization of compound **66** were found in a good agreement with literature reported values of 11α-hydroxy muzigadiolide [85].

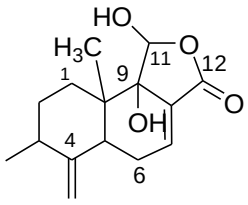
Table 10. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data (acetone-d6) of compound **66** and NMR data (300 MHz, $CDCl_3$) reported for 11*α*-hydroxymuzigadiolide.

	Experimental data of compound **66**		Literature data of 11*α*-hydroxy muzigadiolide	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR [85]
1	31.1	1.43 (1H, *m*)	31.0	1.42 (1H, *m*, H eq)
		2.37 (1H, *m*)		2.55 (1H, *m*, H ax)
2	32.4	1.72 (1H, *m*)	32.2	1.72 (1H, *m*, H ax)
		1.20 (1H, *m*)		1.22 (1H, *m*, H eq)
3	38.4	2.19 (1H, *m*)	38.3	2.09 (1H, *m*)
4	152.6		130.5	
5	40.2	2.68 (1H, *m*)	40.2	2.62 (1H, *m*)
6	26.9	2.3 (2H, *m*)	27.5	2.34-2.42 (2H, *m*)
7	138.3	6.31(1H, *dd*, 0,8, 1.2)	141.4	7.12 (1H, *m*)
8	130.9		130.5	
9	75.4	5.04 (1H*, br s,* OH)	76.5	
10	40.1		53.7	
11	98.2	6.90 (1H, *s*), 5.7(OH	98.6	6.17 (1H, *br s, OH*)

12	166.4		167.3	
13	104.9	4.80 (1H, *s*)	105.9	4.74 (1H, *s*)
		4.90 (1H, *s*)		4.92 (1H, *s*)
14	18.1	1.08 (3H, *d*, 6.8)	18.5	1.09 (3H, *d,* 6.4)
15	14.5	0.77 (3H, *s*)	15.2	0.75 (3H, *s*)

Chemical shifts are reported in parts per million (acetone-d6), ***J*** values are in Hertz.

The structure of compound **66** was proposed based on the ^{1}H and ^{13}C-NMR data comparison with the literature values of 11*α*-hydroxy muzigadiolide and structurally related compound salutarisolide. The quaternary carbon of exocyclic olefinic group (C-4) appeared at *δ* 152.6 in the ^{13}C-NMR of compound **66**, which seemed right position, unlike with the literature values reported at *δ* 130.5 [85]. But, the exact appearance of this carbon, which was confirmed with exocyclic double bond-containing isolated compound **48** in the course and a structurally similar previously isolated compound called salutarisolide from *Warburgia salutaris* [119], would be at *δ* 152.6. So, on the basis of this information,11*α*-hydroxy muzigadiolideis assigned to compound **66**.



66

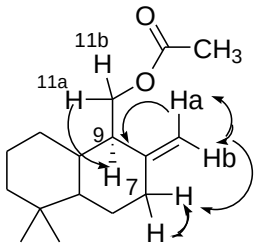
Compound 115

Compound **115** (100 mg) was isolated as a jelly like material from column chromatographic fractionation of the EtOAc soluble portion of the EtOH extract of bark of *W. ugandensis* with hexane:EtOAc as eluent. The TLC profile (Figure 13) of this compound was developed using hexane:EtOAc (5:1) solvent system and 1% vanillin as a spraying agent and showed a single purple spot (Rf 0.5).

The UV-Vis spectrum of the compound showed no absorption band from 600-200 nm indicative of the absence of conjugated chromophore. The IR spectrum of compound **115** exhibited bands at 2923 (C-H stretching), 1735 (acetate unit). The other band observed at

1641 cm^{-1} is due to the presence of exocyclic double bond C-C stretching. Geminal dimethyl stretching is observed at 1376 cm^{-1}. The signal appeared at 1230 cm-1 is due to C-O stretching in the molecule.

The ^{1}H-NMR spectrum of compound **115** displayed four terminal methyl protons each appeared at *δ* 0.71 (3H, *s*), *δ* 0.77 (3H, *s*), *δ* 0.84 (3H, *s*) and *δ* 2.07 (3H, *s*). The two most downfield singlet signals appeared at *δ* 4.87 (1H, *s*) and *δ* 4.53 (1H, *s*) were indicative for the presence of exocyclic double bond (H-12). The other downfield signals appeared as a doublet of doublet at *δ* 4.20 (1H, *dd*, *J* = 11.2, 9.2 Hz) and *δ* 4.34 (1H, *dd*, *J* = 11.2, 3.6 Hz) were due to the diastereotopic oxygenated protons (H-11). The HH-COSY experiment revealed the presence of long-range COSY correlation between the methelene exocyclic double bond protons and an alphatic resonance at *δ* 2.06, assigned for H-7. The H7 resonance showed additional COSY correlations to a resonance at *δ* 2.41, assigned to its geminal H7'. The protons were assigned to the carbon with the help of HSQC and HMBC. The oxygenated protons were only correlated with protons appeared at *δ* 2.07, assigned for H-9.



The ^{13}C-NMR spectrum together with DEPT-135 (Appendix 7) revealed the presence of four methyls, seven methylene, two methine and four quaternary carbon atoms. The downfield signal resonating at *δ* 171.4 together with a methyl signal appeared at *δ* 21.1 was evident for the presence of an ester functional group. The signals observed at *δ* 146.8 and *δ* 107.1 in consistency with the proton NMR experiment were suggestive for the presence exocyclic double bond. The methylene signal appeared at *δ* 61.5 was evident for the presence of one oxygenated carbon. The remaining methylene signals appeared at *δ* 39.0, *δ* 19.1, *δ* 41.9, *δ* 23.9 and *δ* 37.6. The two methine carbons appeared at *δ* 55.0 and *δ* 54.7.

The HSQC spectral data showed that the protons resonating at *δ* 4.53 and 4.87 (each 1H), 4.2 and 4.3 (each 1H), 1.13, 2.09, were correlated with the carbons at *δ* 107.1, 61, 55.0, 54.7 respectively. Close inspection of the HSQC spectrum also showed correlation between protons at *δ* 1.41& 1.20 (each 1H), 1.21 & 1.76 (each 1H) with carbons at *δ* 41.9, 39.04 respectively. In the HMBC spectrum of compound **115**, the methine carbon signal at *δ* 54.7 correlated with protons at *δ* 4.2 and 4.3 (H-9), 4.53 and 4.87 (H-12), 1.23 (1H, H-1). Both of the methylene protons (H11, *δ* 4.2, 4.3) attached to the carbon at *δ* 61.5 (C-11) showed HMBC correlation to the carbons at *δ* 54.7 (C-9), 38.9 (C-10) and 146.8 (C-8).

The HMBC spectrum again revealed the correlation between a carbon signal at *δ* 55.0 with one of the protons of H-7 appeared at *δ* 2.41. This carbon is also correlated with protonsof H-6, H-13 and H-14. In the inspection of HMBC spectrum, there is a close correlation between a corbon signal at *δ* 107.1 with protons of H-7 and H-9.

Both ^{1}H and ^{13}C spectral data of this compound were found in a good agreement with the literature report of albicanyl acetate [120].

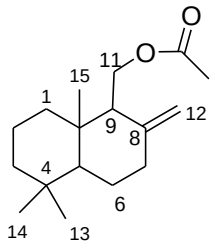
Table 11. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **115** and NMR data (500 MHz, $CDCl_3$) reported for albicanyl acetate

	Experimental data of compound **115**		Literature data of albicanyl acetate [120]	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	39.0	1.25 (1H, *m*), 1.68 (1H, *m*)	39.0	1.25 (1H, *m*, H-eq), 1.68 (1H,
2	19.1	1.45, 1.52 (2H, m, *qt*)	19.2	*m*)
3	41.9		41.9	1.45, 1.52 (2H, *m*, *qt*)
4	33.9		33.5	
5	55.0	1.09 (1H, *m*)	55.1	
6	23.9		23.9	1.09 (1H, *m*)
7	37.6	2.06 (1H, *m*), 2.41 (1H, *qd*)	37.8	
8	146.8		146.8	2.06 (1H, *m*), 2.36 (1H, *m*)
9	54.7	2.09 (1H, *m*)	54.7	
10	38.9		38.9	2.09 (1H, *m*)
11	61.5	4.2 (1H, *dd*), 4.3 (1H, *dd*)	61.6	

12	107.1	4.53 (1H, *s*), 4.87 (1H, *d*)	107.1	4.14 (1H, *dd*), 4.29 (1H, *dd*)
13	33.6	0.84 (3H, *s*)	33.6	4.47 (1H, *s*), 4.81 (1H, *s*)
14	21.7	0.77 (3H, *s*)	21.7	0.84 (3H, s)
15	15.1	0.71 (3H, *s*)	15.1	0.77 (3H, s)
16	171.4		171.4	0.71 (3H, s)
17	21.1	2.07 (3H, s)	21.1	2.07 (3H, s)

s = singlet, *dd* = doublet of doublet, *qd* = quartet of doublet, *m* = multiplet

Comparison of the NMR spectral data of compound **115** with the literature report for albicanyl acetate was in a close agreement (Table 11).



115

This compound was previously reported from marine sponges *Cadlina luteomarginata* [120]. But as to my knowledge, it is reported from the species of Warburgia for the first time.

Compound 49 and 116

Compounds **49** and **116** (20 mg) were obtained as a mixture. The TLC profile of these compounds developed using hexane:EtOAc (4:1) solvent system showed a single violet spot (Rf 0.65) after spraying with 1% vanillin in H_2SO_4 (Figure 13). The IR spectrum of compound **49** together with compound **116** showed a band at 2924 due to C-H stretching. The other bands observed at 1726 and 1675 cm^{-1} are assigned for the presence of α, β unsaturated carbonyl group in the molecule.

The ^{1}H-NMR spectrum of these compounds (Appendix 8) showed four aldehydic protons appeared from *δ* 9.45 upto *δ* 9.54. From these aldehydic protons, two were observed as doublets and the other two appeared as singlet protons. The downfield signal integrated for two protons appeared at *δ* 7.15 was an indicator for the presence of two olefinic

methine protons. The other two protons each appeared as a singlet at δ 4.92 and δ 4.74 were assigned for exocyclic protons. The ^{1}H-NMR spectrum displayed the presence of five terminal methyl protons observed from δ 0.75-1.11.

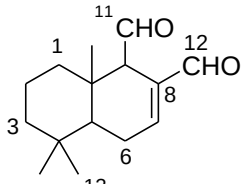
The ^{13}C-NMR spectrum together with DEPT-135 displayed well resolved 30 carbon signals. These were observed as five methyl, eight methylene, eleven methine and six quaternary carbons. The signals displayed at δ 201.8, δ 201.1, δ 193.2 and δ 193.1 were suggestive for the presence of four aldehydic carbons. The ^{13}C-NMR spectrum clearly showed the presence of six olefinic carbons which were appeared at δ 154.3, δ 153.0, δ 151.3, δ 138.2, δ 137.8 and δ 106.0.

The ^{1}H-NMR signals observed from δ 9.45 up to δ 9.54 integrated for four aldehydic protons together with 30 well resolved ^{13}C-NMR signals, from which 15 carbon signals were slightly more intensive than the remaining (Appendix 8), are suggestive that the substance is mixtures of two dialdehydic sesquiterpenes. The ^{13}C-NMR signals appeared at δ 151.3 and δ 106.0 is indicative that there is one exocyclic double bond in one of the compounds from the mixture. The down field signals in the ^{13}C-NMR spectrum appeared at δ 154.3, 152.2, 138.2 and 138.0 together with a proton signal observed at δ 7.14 integrated for two protons are suggestive for the presence of two α, β unsaturated double bonds. It gives a clue that each compound of the mixture is oriented with α, β unsaturated double bond. The efforts made to separate these mixtures into their pure form using PTLC of different solvent system was unsuccessful. As it is indicated in the ^{13}C-NMR spectrum of these compounds, fifteen carbon signals which were appeared slightly intensive than the remaining were identified and compared with previously isolated compounds from this plant and the data agrees with literature reports of 9-deoxymuzigadial. In the same way the remaining fifteen carbon signals were also identified and compared with literature reports of polygodial as it is depicted in table 12.

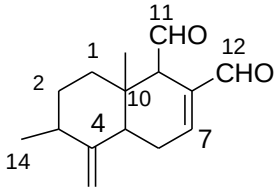
The ^{1}H and ^{13}C NMR spectrum data of these compounds were compared with literature reports of polygodial and 9-Deoxymuzigadial and found in a good agreement [121, 122].

Table 12. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **49** and **116,** and NMR data reported for polygodial (400 MHz, $CDCl_3$) and 9-Deoxymuzigadial (200 MHz, $CDCl_3$)

	compound **49**			Compound **116**		
	^{13}C-NMR	^{1}H-NMR	Lit [122]	^{13}C-NMR	^{1}H-NMR	Lit [121, 122]
1	39.5	1.37, 1.82 (2H, *m*)	39.8	39.3	1.62, 1.91(2H, *m*)	39.4
2	18.0	1.48, 1.60 (2H, *m*)	18.0	31.5	1.71, 1.15 (2H, *m*)	31.6
3	41.0	1.19, 1.48 (2H, *m*)	42.0	38.4	2.10 (2H, *m*)	38.5
4	33.1		33.0	151.3		151.3
5	48.9	1.25 (1H, *m*)	49.0	45.7	2.1 (1H, *m*)	45.8
6	25.2	2.49, 2.32 (2H, *m*)	25.3	26.9	2.45 (2H, *m*)	27.0
7	154.3	7.14 (1H, *m*)	152. 2	153.0	7.14 (1H, *m*)	152.9
8	138.2		138.0	137.8		137.9
9	60.2	2.83 (1H, *m*)	60.0	58.2	3.01 (1H, *m*)	58.3
10	37.0		37.0	38.1		38.2
11	202.0	9.54 (1H, *d*)	202.0	201.2	9.52 (1H, *d*)	201.1
12	193.2	9.5 (1H, *s*)	194.0	193.3	9.46 (1H, *s*)	193.3
13	33.1	0.93 (3H, *s*)	33.0	106.0	4.92 (1H, *s*), 4.74 (1H, *s*)	106.0
14	21.9	0.95 (3H, *s*)	22.0	18.40	1.01 (3H, *d*)	18.4
15	15.2	1.09 (3H, *d*)	15.0	13.54	0.74 (3H, *s*)	13.5



49



116

Polygodial (**49**) was first reported from and named after, the water pepper *Polygonum hydropiper* [123]. The previous report indicated that these two compounds exhibited highly antifeedant activity and are most widely occurring sesquiterpene dialdehydes, having been found in flowering plants, marine sponges, ferns and liverworts [123, 124].

Compound 117

Compound **117** (20 mg) was obtained as abrown jelly material from repeated column chromatographic fractionation of the EtOAc soluble portion of the EtOH extract of bark of *W. ugandensis* with hexane:EtOAc of increasing polarity as eluent. The TLC profile of the compound developed using hexane:EtOAc (4:1) solvent system showed single spot with Rf values of 0.5 after spaying with 1% vanillin in sulfuric acid.

The UV-Vis spectral analysis showed the presence of conjugated chromophore with λmax 217, 298 nm, indicating the presence of a benzoid moity. In the IR spectrum of compound **117**, the broadband observed at 3429 cm^{-1} is suggestive of the presence of an OH group in the molecule. The sharp peak appeared at 2916 is due to the C-H stretching. The peaks at 1607 and 1478 cm^{-1} are indicative of the presence of the aromatic ring. The other band displayed at 1376 cm^{-1} is a characteristic for the presence of geminal dimethyl stretching. The peak at 1205 cm^{-1} is due to the C-O stretching.

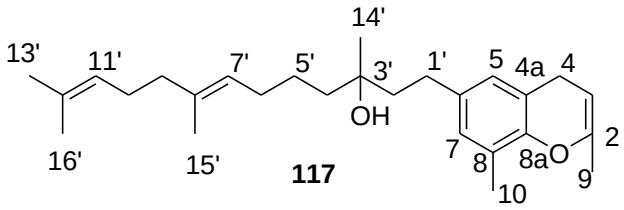
The aromatic regions of the ^{1}H-NMR spectra (Appendix 9) showed two *meta* coupled protons at *δ* 6.49 (1H, *d*) and *δ* 6.40 (each 1H, *d, J = 3.2*). Furthermore, the presence of other olefinic protons is confirmed by the proton signals observed at *δ* 5.08-5.14 (3H, *m*). The triplet signal appeared at *δ* 2.71 integrated for two protons is due to two methylene protons of chain appended to an aromatic ring. In the ^{1}H-NMR spectrum, six terminal methyl protons are observed of which three methyl protons are appeared being overlapped at *δ* 1.61 (9H, *s*). The remaining terminal methyl protons were shown at *δ* 1.70 (3H, s), *δ* 1.28 (3H, *s*) and *δ* 2.14 (3H, *s*).

In the ^{13}C-NMR spectrum (Appendix 9), the carbon signals are observed in the olefinic (aromatic) and aliphatic regions. There are twelve carbon signals observed in the olefinic region from *δ* 112.6 up to *δ* 147.8. Only five of them (*δ* 112.6, *δ* 115.7, *δ* 124.2, *δ* 124.3 and *δ* 124.4) are methine carbons and the rest seven (*δ* 121.1, *δ* 127.2, *δ* 131.2, *δ* 134.9, *δ* 135.1, *δ* 145.9 and *δ* 147.8) are quaternary carbons. The ^{13}C-NMR spectrum together with DEPT-135 showed one oxygenated quaternary carbon at *δ* 75.3. All NMR data are compared with the literature values of caseamemic previously isolated from the stem of *Casearia membranacea* [125] and found in a good agreement.

Table 13. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **117** and NMR data (400 MHz, $CDCl_3$) reported for caseamemic

Experimental data of compound **117**			Literature data of caseamemic [125]	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2	147.8		147.7	
3	124.2	5.12 (1H, *m*)	124.2	5.13 (1H, *m*)
4	22.2	2.14 (2H, *m*)	22.1	2.14 (2H, *m*)
4a	134.9		135.0	
5	112.6	6.40 (1H, *d*, *J* =2.8Hz)	112.6	6.38 (1H, *d*, *J* = 3 Hz)
6	121.2		121.2	
7	115.7	6.49 (1H, *d, J* = 2.8 Hz)	115.6	6.48 (1H, *d*, *J* = 3 Hz)
8	127.3		127.4	
8a	145.9		146.0	
9	16.0	1.62 (3H, *s*)	16.0	1.60 (3H, *s*)
10	15.9	2.14 (3H, *s*)	15.9	2.13 (3H, *s*)
1’	22.5	2.71 (2H, *t*, *J* = 6.4 Hz)	22.5	2.69 (2H, *t*, *J* = 6.6 Hz)
2’	31.4	1.74 &1.81 (2H, *td*)	31.3	1.74 &1.81 (2H, *td*)
3’	75.3		75.3	
4’	39.7		39.7	1.53 & 1.64 (2H, *m*)
5’	26.7	2.01 (2H, *m*)	26.7	2.04 (2H, *m*)
6’	39.7	1.75 (2H, *m*)	39.7	1.69 (2H, *m*)
7’	124.3	5.12 (1H, *m*)	124.3	5.08 (1H, *m*)
8’	135.1		135.1	
9’	39.7	1.78 (2H, *m*)	39.7	1.69 (2H, *m*)
10’	26.6	2.09 (2H, *m*)	26.6	2.08 (2H, *m*)
11’	124.4	5.12 (1H, *m*)	124.4	5.10 (1H, *m*)
12’	131.3		131.3	
13’	25.7	1.61 (3H, *s*)	25.7	1.59 (3H, *d*, *J* = 0.8 Hz)
14’	24.0	1.28 (3H, *s*)	24.0	1.26 (3H, *s*)
15’	16.0	1.62 (3H, *d*)	16.0	1.60 (3H, *s*)
16’	16.7	1.70 (3H, *s*)	17.7	1.68 (3H, *d*, *J* = 0.8 Hz)

Comparison of the NMR spectral data of compound **117** with the literature reported for caseamemic was in close agreement (Table 13).



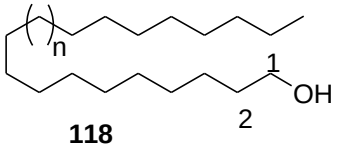
117

This compound was isolated before from the stem of *Casearia membranacea*. It is cytotoxic. To the best of our knowledge it has not been reported before from the Warburgia species.

Compound 118

Compound **118** (10 mg) was obtained as oil like jelly material from EtOAc soluble portion of the EtOH extract of bark of *W. ugandensis*. It was recrystallized from hexane as a fine powder which melts at 74-75^{o}C. The compound showed a single purple spot on TLC (Rf 0.68) after it developed using hexane:EtOAc (4:1) solvent system as mobile phase and vanillin as a spraying agent (Figure 13).

The UV-Vis spectrum of compound **118** displayed visible bands neither in the UV nor in the visible region indicating the absence of conjugated chromophore in the structure. The IR spectrum displayed signals at 3395 cm^{-1} for O-H stretching, 2924, 1470 for C-H stretching and bending and 708 for C-C stretching. The ^{1}H-NMR spectrum (in $CDCl_3$, Appendix 10) demonstrated a triplet signal at δ 3.66 (J=6.4 Hz) assigned to methylene protons on oxygenated carbon. The quintet signal at δ 1.58 is due to methylene protons on carbon flanked between two methylene groups. A broad signal at δ 1.27 (48H) is a characteristic signal for several overlapping methylene protons. The presence of terminal methyl group in the compound is confirmed with an upfield triplet signal appeared at δ 0.90 (3H, *J*=6.7 Hz). The ^{13}C-NMR spectrum (Appendix 20) showed a downfield signal at δ 63.1 corresponds to an oxygenated methylene carbon. Furthermore, the carbon signal observed at δ 14.1 is a characteristic signal for terminal methyl groups. The ^{13}C-NMR data with the help of DEPT-135 indicative the compound is a straignt chail alcohol. The number of methylene groups may be revealed by generation of MS data.

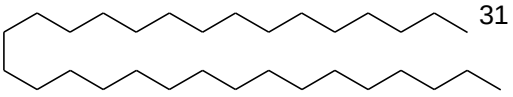


118

Compound 119

Compound **119** was isolated from column fractions (fraction one) of $CHCl_3$ extracts of leaves of *W.ugandensis* as a white amorphous solid (50 mg). The TLC profile of the compound developed using hexane:$CHCl_3$(3:2) solvent system as a mobile phase and vanillin as a spraying agent showed pink color single spot (Rf 0.75). The compound melts at 58-59^oC (lit. 67-68^oC)[126]. In the mass spectrum, compound 119 displayed *m/z*-at 436 which establish the molecular formula of the compound as $C_{31}H_{64}$. The UV-Vis spectrum (in ethanol) showed no absorbance from 600-200 nm which indicates the absence of conjugated chromophore. The IR spectrum showed absorption band at 2914 and 1470 cm^{-1} due to C-H stretching and bending respectively. The other band observed at 718 $cm^{-1}$is evident for the presence of C-C stretching.

The ^{1}H-NMR spectrum (Appendix 11) showed signals at δ 0.90 (6H, *t*) and at δ 1.27 (*br s*) were suggestive the compound is normal chain alkane. The ^{1}H-NMR data was supported by ^{13}C-NMR and DEPT-135 with only six carbon signals. From the six carbon signals, five were methylene at δ 31.9, 29.7, 29.6, 29.3 and 22.7 and one methyl at δ 14.1. The 1H and ^{13}C-NMR spectral suggest straight-chain alkane. The MS and NMR data confirmed that compound **119** is hentriacontane. This compound was isolated from EtOAc extract of *Oryza sativa* (rice) [127] and hexane extracts of *Gymnosperma glutinosum* (Asteraceae) leaves [128].



119

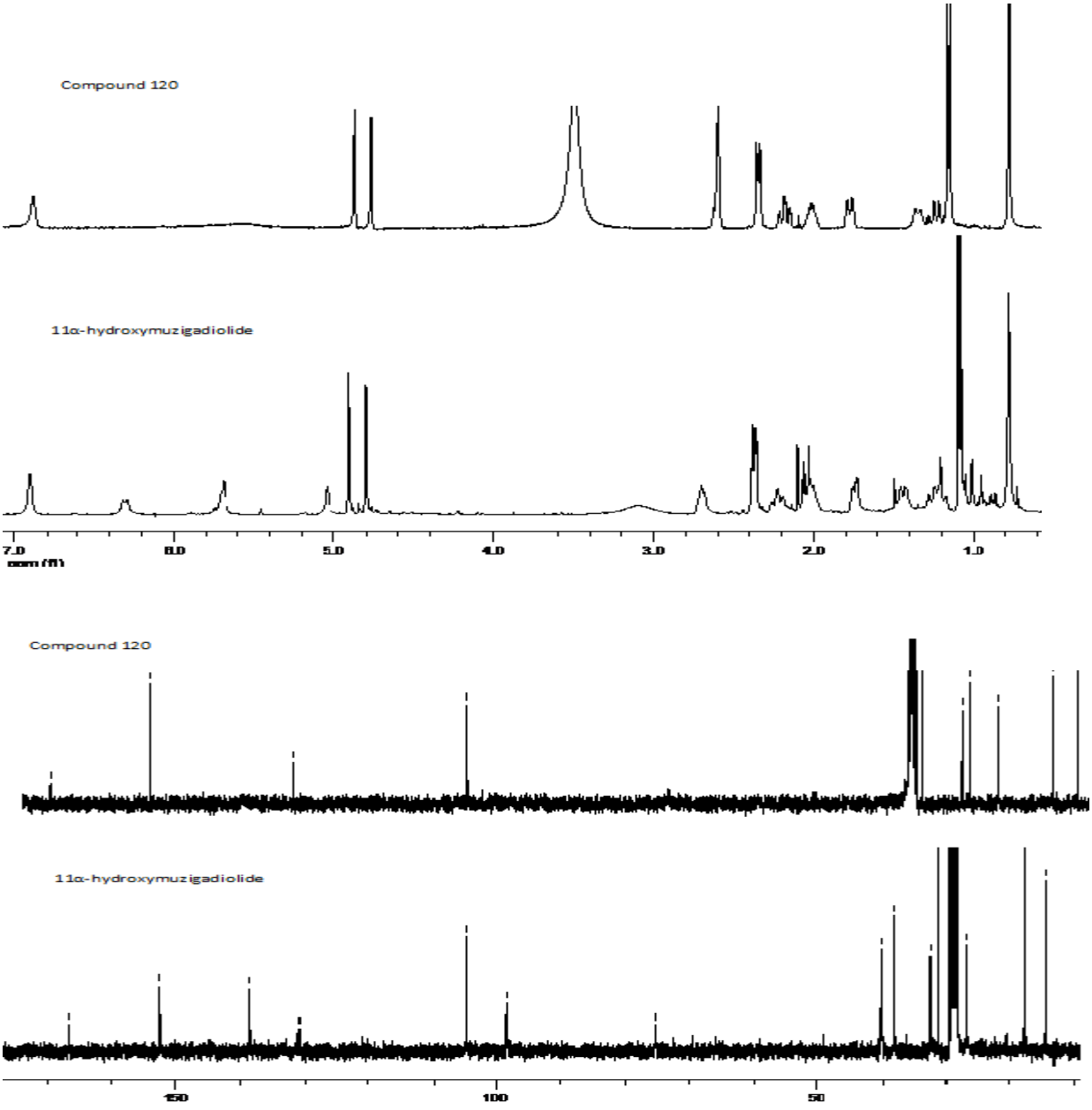
Compound 120

Compound **120** (30 mg) was obtained as a white powder from column chromatographic fractionation of ethyl acetate soluble portion of the ethanol extract of the bark of *Warburgia ugandensis*. The melting point of the compound was found 140-142^{o}C.The TLC profile of the compound was studied using hexane:EtOAc (1:4) solvent system as a mobile phase and vanillin in H_2SO_4 as a spraying agent and a single spot (Rf 0.40) was observed.

The UV-Vis spectrum showed absorbance maxima (λ_{max} in EtOH) at 220 nm due to the transition of electrons from π-π* indicative the presence of conjugated chromophore. (+)-ESI MS exhibited an ion peak $[M+Na]^+$ at *m/z* = 287 ($C_{15}H_{20}NaO_4$), at [M+H]+ at *m/z* = 265 ($C_{15}H_{21}O_4$) and $[M+NH_4]^+$ at *m/z* = 282 ($C_{15}H_{24}NO_4$) confirming the molecular formula of the compound is $C_{15}H_{20}O_4$. The IR spectrum of the compound revealed a broadband peak at 3393 cm^{-1} indicating the presence of OH stretching associated with hydrogen bridges. The presence of a sharp peak at 1709 cm^{-1} is evident for the presence of carbonyl group in the molecule. The peaks appeared at 2968, 1171 and 1636 cm^{-1} were assigned for C-H, C-O, and C=C stretching respectively.

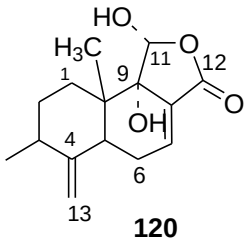
The ^{1}H-NMR spectrum of compound **120** (Appendix 12) together with HSQC showed the presence of two protons each appeared as a singlet at *δ* 4.73 and *δ* 4.83 correlated with a carbon appeared at *δ* 105.82 indicator of the presence of an exocyclic double bond. The signals appeared at *δ* 1.02 (3H, *d*) and *δ* 0.64 (3H, *s*) were an indicative for the presence of two terminal methyl groups of which one attached with a methine carbon and the other with a quaternary carbon. A proton signal observed at *δ* 2.50 (1H, *m*) was assigned with the help of HSQC as a methine proton of a carbon appeared at *δ* 40.50. The ^{1}H-NMR spectrum together with HSQC showed three different methylene protons appeared at *δ* 2.23 (2H, *dd*), *δ* 2.04 (1H, *ddd*) and *δ* 1.12 (1H, *m*) and *δ* 1.64 (1H, *dd*) and *δ* 1.08 (1H, *dd*). The ^{13}C-NMR together with DEPT-135 confirmed the presence of two methyl (*δ* 15.3 and *δ* 18.9), four methylene (*δ* 27.1, *δ* 31.4, *δ* 32.5 and *δ* 105.8), two methine carbons (*δ* 38.4 and *δ* 40.5) and four quaternary carbons (*δ* 167.5 for carbonyl carbon, *δ* 152.8, *δ* 131.5 and *δ* 75.9). The (+)-ESI MS analysis indicated that the molecular weight and the formula are similar with the compound 11α-hydroxyl muzigadiolide, isolated from the

same fraction. The NMR data are also indicative that the observed ^{1}H and ^{13}C-NMR signals of compound **120** showed closed similarities, except the missed signals in compound **120**, with the ^{1}H and ^{13}C-NMR chemical shift values of 11α-hydroxy muzigadiolide as it is depicted below.



The above spectroscopic data (the molecular formula and weight obtained from MS analysis and NMR signals) indicative that the structure of compound **120** resembles to the structure of 11α-hydroxy muzigadiolide. Compound **120** may be an epimer of 11α-

hydroxy muzigadiolide. The exact structure of compound **120** should be determined using different advanced spectroscopic methods like crystallography.



120

2.3.7. Bioassay results of compounds isolated from *W. ugandensis*

Compounds isolated from *W. ugandensis* bark were studied for their phytotoxicity activity *in vitro* at 0.05 mg/mL concentration against Parthenium weed seed germination. Muzigadial (**48**), ugandensidial (**60**) and cinnamolide-3-β-acetate (**64**) showed the highest activity with 100, 100 and 96% GI respectively (Table 15).

Table 14. *In vitro* bioassay results of pure compounds from *W. ugandensis*

compound	number of germinated seeds					
	T1	T2	T3	mean	STDV	%GI
48	0	0	0	0	0	100
120	4	4	3	3.6	0.57	59
64	3	2	5	3.3	1.5	96
60	0	0	0	0	0	100
66	4	3	2	3	1	66
119	3	4	5	4	1	55
Roundup®	0	0	0	0	0	100
5% acetone	9	9	9	9	0	0

The *in vivo* phytotoxicity study revealed after the treatment of 15-day old seedlings of *P. hysterophorus* weed with compounds **48**, **64**, **60**, **119** and **120** at 1 mg/mL concentration, significant inhibition of growth was observed for all treatments. Interestingly, of compounds isolated from *W. ugandensis* bark in this work, muzigadial (**48**) (Table 16) displayed the highest biological activity to inhibit the growth of the 15 day old test seedlings of parthenium weed (95% GI) *in vivo* with inhibition concentrations (*IC*) of 1 mg/mL (Figure 11). The commercial herbicide roundup® assayed as positive control showed similar inhibition with muzigadial (**48**) at the same concentrations.

When seedlings of *P. hysterophorus* weed were treated with cinnamolide-3*β*-acetate (**64**) and ugandensidial (**60**) at the same concentration, 91% and 87% growth inhibition was observed respectively. The growth of the target weed seedlings treated with compound **120**, 11α-hydroxy muzigadiolide (**64**) and compound **119** showed 66, 40 and 18% growth inhibition respectively.

Table 15. *In vivo* results of pure compounds from *W. ugandensis*

compounds	ADB of seedlings before treatment (mg)				ADB of seedling after treatment (mg)				Mass d/nce	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
48	13	15	8	12	24	8	16	16	4	95
120	13	15	8	12	59	41	29	43	31	66
64	13	15	8	12	20	24	16	20	8	91
60	13	15	8	12	24	25	20	23	11	88
119	13	15	8	12	84	81	93	86	74	19
Roundup®	13	15	8	12	14	16	18	16	4	95
5% acetone	13	15	8	12	98	109	103	103.3	91.3	0

In the efforts we made to find herbicidal compounds from plants against *P. hysterophorus* weed, Muzigadial on the basis of its phytotoxic screening test result, which was done in the greenhouse in comparison with the standard commercial herbicide Roundup®, can be one of the contribution of this work to be used as a future generation of herbicide in the management of parthenium weed in addition to dehydrocostus lactone from *E. kebericho*. The previous study indicates that muzigadial displayed pronounced antimicrobial activities which revealed that the dialdehydes moiety was responsible for the broad range of antimicrobial activity of muzigadial [64, 84]. The presence of aldehyde functional groups may also be responsible for the phytotoxic activity isolated compounds exhibited.

Table 16. Summary of *In vitro* and *in vivo* results of pure compounds from *W. ugandensis*

Compound name	*% GI (In vitro)* 0.05 mg/mL	*%GI (In vivo)* 1 mg/mL
Muzigadial (**48**)	**100**	**95**
Cinnamolide-3*β*-acetate(**64**)	96	91
ugandensidial (**60**)	100	87
11α-hydroxy muzigadiolide (**66**)	66	40
compound **120**	59	66
compound **119**	55	18
roundup® (+ve control)	**100**	**97**
5%acetonein water (-ve control)	0	0

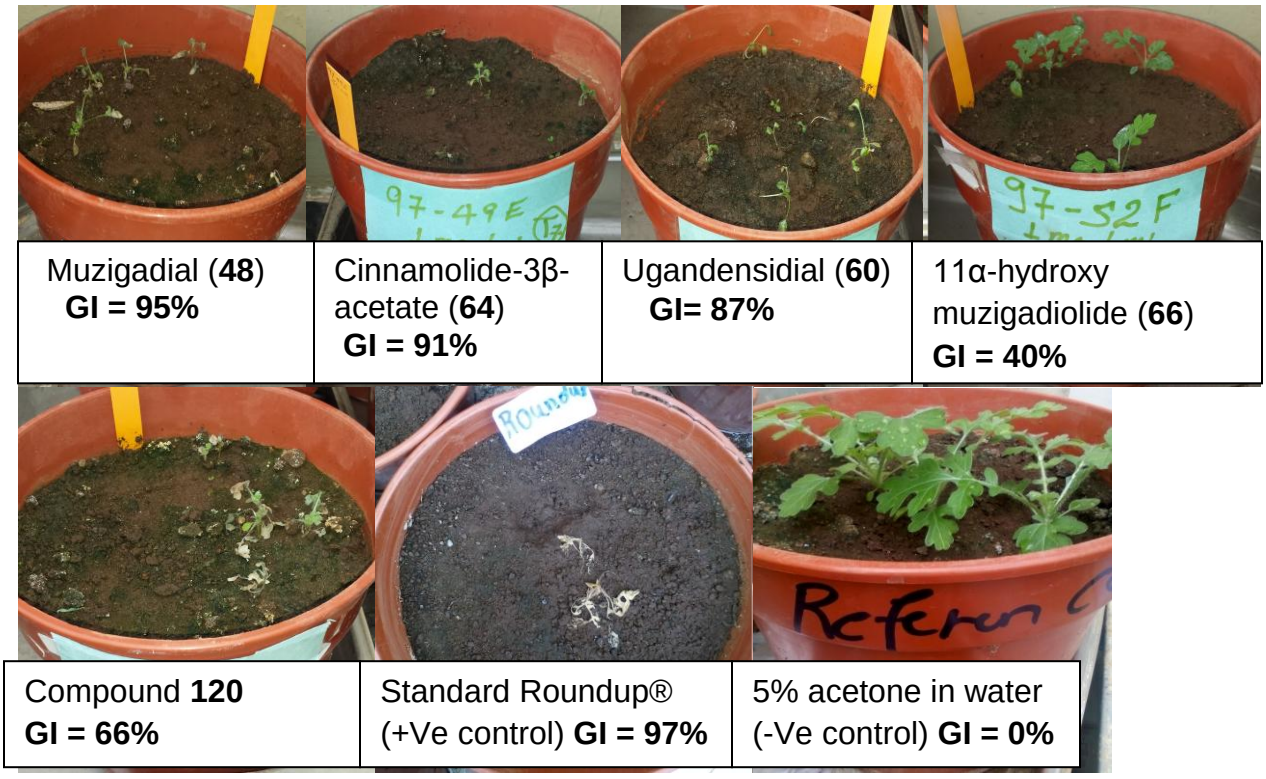


Figure 14. *In vivo* bioassay results of compounds from *W. ugandensis* in greenhouse

2.3.8. Isolation of compounds from *A. aspera*

Aerial parts of A. aspera (100 g) was extracted with ethanol and concentrated with rotary vopourator to furnished 4 g (4%) crude extract. This was applied over silica gel column chromatography and resulted in the isolation of five compounds such as stigmasterol (82), stigmasterol glycoside (121), sitosterol-glycoside (122), Linolenic acid (123) and sucrose (124). The TLC of compounds isolated from ths plant is depicted as follows.

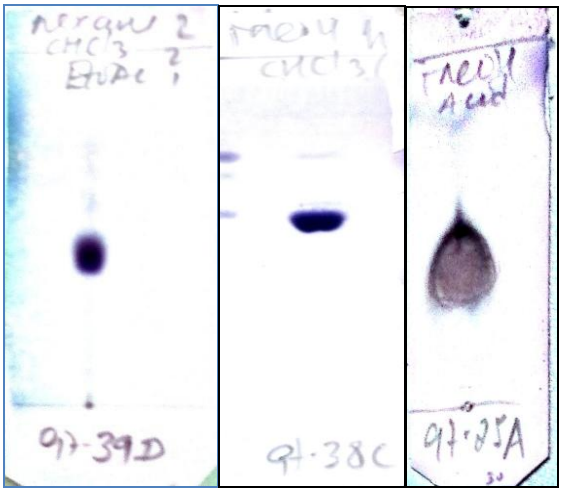


Figure 15. TLC profile of sitigmasterol, stigmasterol-glycoside, sitosterol-glycoside

2.3.9. Characterization of compounds isolated from *A. aspera*

Compound 82

Compound **82** (20 mg, 0.6%) was obtained from fraction 5 as a white solid material melting at 140-145^{o}C (Lit. 174-176^{o}C) [117]. The TLC profile developed using hexane:$CHCl_3$:EtOAc (2:2:1) as a mobile phase was visualized as purple color single spot (Rf 0.82) after spraying with vanillin in H_2SO_4 and heating with a hot air gun (Figure 15).

The UV-Vis spectrum (in EtOH) showed no absorption which indicates there is no any conjugated chromophore. The IR spectrum showed absorption at 3430 cm^{-1} indicative of the presence of O-H stretching. Other picks appeared at 2938 and 1463 cm^{-1} were interpreted to C-H stretching and bending of the methyl group. In the IR spectrum the C-O stretching appeared at 1050 cm^{-1}.

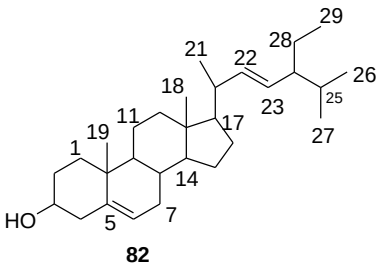
^{1}H-NMR spectrum (Appendix 13) of compound **82** revealed the presence of three olefinic protons each appeared at *δ* 5.03 (1H, *m*), *δ* 5.16 (1H, *m*) and *δ* 5.36 (1H, *t*). The proton corresponding to the H-3 of a sterol moiety at C-3 appeared as a triplet of doublet of doublets at *δ* 3.51.

The ^{13}C-NMR spectrum of compound **82** showed 29 carbon signals including 6 methyls, nine methylene, eleven methine and three quaternary carbons. Of the 11 methine carbons, the signals appeared at *δ* 138.3, *δ* 129.2 and *δ* 121.7 were assigned to olefinic carbons (C-22, C-23, and C-6) respectively. The signal at 71.8 is due to an oxygenated carbon (C-3). The downfield signal appeared at *δ* 140.7 is an indication to the olefinic quaternary carbon(C-5). The NMR spectral data of compound **82** was compared with literature values of stigmasterol [117] and found in a good agreement (Table 18). This compound was previously reported from the root of *A. aspera* [129].

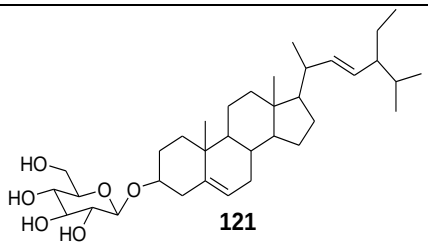
Table 17. ^{13}C-NMR spectral data comparison of compounds 70, 109 and 110 with literature reported for stigmasterol, stigmasterol glycoside and β-sitosterol glycoside respectively

	82 ($CDCl_3$, *δ* in ppm)		**121** (DMSO, *δ* in ppm)		**122** (DMSO, *δ* in ppm)	
NO	^{13}C-NMR	Lit. [117]	^{13}C-NMR	Lit. [130]	^{13}C-NMR	Lit. [131]
1	37.2	37.6	37.3	37.3	37.3	36.8
2	31.9	32.1	31.9	31.9	31.8	29.1
3	71.8	72.1	77.4	77.4	77.3	78.6
4	42.3	42.4	42.2	39.6	42.3	42.1
5	140.7	141.1	140.9	140.9	140.9	139.9
6	121.7	121.8	121.7	121.7	121.6	121.5
7	31.6	31.8	29.7	29.7	31.8	31.4
8	31.9	31.8	31.8	31.9	31.9	31.4
9	50.1	50.2	50.1	50.1	50.0	49.8
10	36.5	36.6	36.7	36.7	36.7	36.2
11	21.1	21.5	21.4	21.6	20.2	20.2
12	39.7	39.9	38.7	38.8	38.7	38.2
13	42.2	42.4	42.3	42.2	42.2	41.8

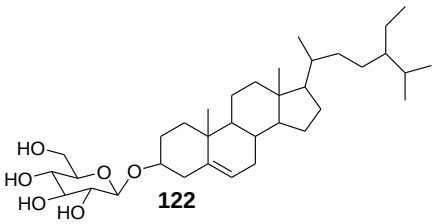
14	56.8	56.8	56.6	55.8	56.6	56.3
15	24.3	24.4	24.3	24.4	24.3	23.7
16	29.1	29.3	29.1	29.0	29.2	27.7
17	56.0	56.2	55.9	57.7	55.9	55.6
18	12.2	12.2	12.1	12.3	12.1	11.2
19	19.0	18.9	19.3	19.3	19.5	19.1
20	40.5	40.6	45.6	40.0	35.9	35.7
21	21.2	21.7	20.21	21.4	19.4	18.6
22	138.3	138.7	139.5	138.5	33.8	33.5
23	129.2	129.6	129.3	129.3	25.3	25.6
24	45.8	46.1	51.0	51.1	45.6	45.4
25	28.2	29.6	31.9	31.8	29.7	28.7
26	19.8	20.2	19.5	21.1	19.3	18.6
27	19.4	19.8	19.4	19.6	19.1	18.3
28	25.4	25.4	25.3	25.5	23.1	22.6
29	12.0	12.1	12.2	12.6	12.2	12.2
1’			101. 3	101.2	101. 2	100.7
2’			73.9	73.9	73.5	73.21
3’			76.7	77.2	77.1	76.1
4’			70.5	70.6	70.5	69.9
5’			77.1	77.0	77.3	75.6
6’			61.5	61.6	61.5	61.3



82



121



122

Compound 121

Compound **121** (20 mg, 0.6%) was obtained from subfraction four of fraction 8 using EtOAc:MeOH (9:1) as eluent. Fraction 8 (150 mg) was applied on Sephadex LH-20 and compound **121** was obtained as a white solid material from fraction 4. The compound melts at 260-262^{o}C (lit. 278-280°C) [132] which indicates the presence of some interferences. The TLC profile (Figure 15) developed using formic acid:EtOAc:MeOH (1:2:3) as a mobile phase was visualized as a single spot (Rf 0.61) after spraying with vanillin in H_2SO_4 and heating with a hot air gun.

The UV-Vis spectrum (in EtOH) showed no absorption band which indicated there is no any conjugated chromophore. The IR spectrum showed a broad absorption at 3428 cm^{-1} is an indicator of the presence of O-H stretching. The pick at 1625 cm^{-1} is a characteristic peak for unsaturation for C=C. Other picks appeared at 2916 and 1023 cm^{-1} were due to C-H of Methyl group and C-O stretching respectively.

The interferent signals observed in the ^{1}H-NMR spectrum of compound **121** (Appendix 14) were indicative the compound is not pure. But, the the interpretation of this compound is was done using the more intensive signals in the spectrum. The presence of a signal at *δ* 5.33 (1H, d) evident for the olefinic proton of a stigmasterol (H-6). Other downfield signals appeared at *δ* 5.33 (1H, *dd*, H-22) and *δ* 5.02 (1H, *dd*, H-23) were assigned for the external double bond (out of the ring) of a sterol moiety. In the ^{1}H-NMR spectra, the presence of six terminal methyl protons appeared at *δ* (0.50 (3H, *s*, H-18), *δ* 0.80 (3H, *d*, H-27), *δ* 0.82 (3H, *d*, H-26), *δ* 0.89 (3H, *d*, H-29), *δ* 0.96 (3H, *s*, H-19) and *δ* 0.99 (3H, *d*, H-21) were observed. A doublet signal for a proton at *δ* 4.21 (1H, *d*) was evident for an anomeric proton of the sugar moiety. The proton signals observed from *δ* 4.87-4.92 were assigned for the sugar OH protons. The methylene protons of the sugar (H-6) appeared as a multiplet at *δ* 3.62 (2H, *m*). As it is shown in the ^{1}H-NMR spectrum (Appendix 14), other minor signals are observed in the spectrum indicating compound **121** contaminated with some impurities.

The ^{13}C-NMR spectrum together with DEPT-135 (Appendix 14) showed the marker signals of stigmasterol moiety of sterol glycoside at δ 140.9, 121.7, 138.5 and 129.3 assigned for carbons of C-5, C-6, C-22 and C-23 respectively. A peak observed at *δ*

77.41 was indicative for the presence of an oxygenated carbon (C-3) in the molecule. The presence of glucose moiety within the compound was confirmed by observing six ^{13}C signals appeared at *δ* 61.5, 70.5, 73.9, 76.7, 77.1 and 101.3. The NMR spectral data of this compound were compared with literature values of stigmasterol glycoside [130] and found in a close agreement. So, compound **121** was proposed to be Stigmasterol glycoside.

Compound 122

Compound **122** was isolated as a white powder (10 mg) from the ethanol extracts of the aerial parts of *A. aspera.* The TLC developed using EtOAc:MeOH (4:1) as a mobile phase and vanillin as a spraying agent showed a single broad blue colored spot (Rf = 0.75).

^{1}H-NMR spectrum data demonstrated the presence of olefinic protons at *δ* 5.33 (1H, *t*). The appearance of a proton for the oxygenated carbon at *δ* 4.44 (1H, *m*) along with 6 methyl groups at *δ* 0.65 (3H, *s*), *δ* 0.76 (3H, *d*), *δ* 0.78 (3H, *m*), *δ* 0.80 (3H, *d*), 0.89 (3H, *d*) and *δ* 0.95 (3H, *s*) confirmed for the presence of a sterol moiety with one characteristic double bond. The ^{1}H-NMR spectrum showed an anomeric proton (H-1') of glucose at *δ* 4.21 (1H, *d*) with a *J* value of 8.00 Hz establishing the sugar as a *β*-anomer. The multiplet signals at *δ* 4.89 accounts for hydroxyl groups of the sugar moiety.

The ^{13}C-NMR spectrum (Appendix 15) showed 35 signals. The olefinic carbons were observed at *δ* 140.9 and 121.6. The oxygenated carbon of the sitosterol moiety (C-3) appeared at *δ* 77.2. The pick observed at *δ* 101.2 for the anomeric carbon confirms the presence of only one sugar moiety and the remaining carbons of the sugar appeared at *δ* 77.3, 77.1, 73.5, 70.5 and 61.5. The ^{1}H and ^{13}C-NMR data are correlated with the literature values of sitosterol *3-O-D*-glycoside and found in a close agreement [131]. Based on the ^{1}H and ^{13}C-NMR data and its literature confirmation, the compound is proposed to be sitosterol *3-O-D*-glycoside.

Compound 123

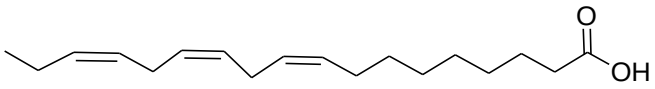
Compound **123** (15 mg) was isolated from subfraction three of fraction six of ethanol extracts of *achyranthes aspera* as a light yellowish powder. The TLC profile of compound

123 developed using $CHCl_3$:EtOAc (1:1) as a mobile phase and vanillin in H_2SO_4 as a spraying agent showed a yellow single spot (Rf 0.24).

The ^{1}H-NMR spectrum (Appendix 16) of compound **123** showed a multiplet downfield signal appeared at *δ* 5.38 indicative of protons at a double bond position. A triplet signal appeared at *δ* 0.99 (slightly more downfield than the terminal methyl protons of saturated fatty acids and non n-3 series of PUFA in triglycerides) is a characteristic signal for terminal homoallylic methyl protons of n-3 polyunsaturated fatty acids (PUFA). The peak observed at *δ* 2.06 is interpreted due to the allylic methylene protons. The signal observed at *δ* 2.35 (2H, *t*) is assigned for methylene protons adjacent to carboxylic acid group. The proton signal at *δ* 1.65 is assigned for the protons of CH_2 group β to the carboxylic acid. The peak observed at *δ* 2.81 (protons attached to the *bis*-allylic carbons) together with the peak at *δ* 0.99 indicative for the presence of n-3 series of polyunsaturated fatty acid in triglycerides [133]. The pattern of proton signals of compound **123** observed in the ^{1}H-NMR spectrum is found in the similar region with the proton spectrum of methyl soyate (methyl ester derived from soybean oil) [134]. The remaining peak observed at δ 1.27 was assigned to the methylene (CH_2) in the fatty acid chain.

The ^{13}C-NMR spectrum showed signals from 14.3 to 33.9 and from 127.1 to 131.9 together from a signal appearing at *δ* 179.2 indicative the compound is polyunsaturated fatty acid. The signals of six methine carbons appeared at *δ* 127.1, 127.7, 128.2, 128.2, 130.2 and 131.9 suggestive the presence of three double bonds within the structure of the fatty acid. In the ^{13}C-NMR, a total of 18 carbons observed and the compound may be 18:3 fatty acids.

The proposed fatty acid is linolenic (9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic acid

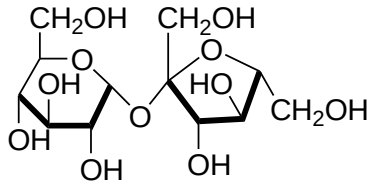


123

Sucrose (124)

Compound **124** (10 mg) was isolated as a white solid from *A. aspera* aerial part. It is soluble in water and hardly soluble in MeOH. The ^{1}H-NMR spectrum of compound **124** displayed signals in the oxygenated region only indicative carbons is bearing hydroxyl groups. The spectrum showed the signal for one anomeric proton at *δ* 5.41(1H, *d, J*= 3.6 Hz). The remaining proton signals of this compound appeared in the region between 3.32 and 4.64.

The ^{13}C-NMR together with DEPT-135 revealed the presence of one quaternary, three methylene and eight methine carbons. The compound exhibited an anomeric signal at *δ* 103.93. The other carbon signals are appeared at *δ* 92.27, 82.35, 77.9, 74.3, 73.2, 73.0, 71.8, 69.9, 62.6, 62.0 and 60.8. All were found in the oxygenated region. The NMR data of compound 66 were compared with the authentic sucrose and showed similar signals.
The TLC of compound **124** and authentic sucrose developed using MeOH:EtOAc: AcOH (3:2:1) as a mobile phase and vanillin as a spraying agent displayed spots with co-spot Rf values.



124

2.3.10. Bioassay results of compounds isolated from *A. aspera* aerial part

Even though more than six compounds were isolated from the plant in the course, only stigmasterol (**82**) and stigmasterol glycoside (**121**) were screened for their herbicidal property both *in vitro* and *in vivo*. Both compounds found to be actively suppressing the seed germination by 100% GI *in vitro* at 0.05 mg/mL concentration.

Table 18. *In vitro* result of compound **82** and **121**

compound	source	number of germinated seeds					
		T1	T2	T3	mean	STDV	%GI
82	*A. aspera*	0	0	0	0	0	100
121		1	0	0	0.33	0.57	95
Roundup®		0	0	0	0	0	100
5% acetone		7	7	7	7	0	0

In vivo herbicidal test of stigmasterol glycoside (**121**) inhibited the 15 days parthenium weed seedling germination by 50 % GI at 1 mg/mL. However, the *in vivo* herbicidal activity test of stigmasterol (**82**) with the same concentration is found insignificant (14% GI). Therefore, the sugar moiety has played a role for the better herbicidal potential of stigmasterol. Herbicidal activity of compound **122**, **123** and **124** was not studied due to a small amount of each compound.

Table 19. *In vivo* results of compound **82** and **121**

compounds	ADB of seedlings before treatment (mg)				ADB of seedling after treatment (mg)				Difference	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
82	28	31	30	30	350	301	279	310	280.3	15
121	28	31	30	30	190	170	180	180	150.3	50
Roundup®	28	31	30	30	43	39	38	40	10.33	97
5% acetone	28	31	30	30	362	360	358	360	330.3	0

Stigmasterol glycoside (**121**) (GI = 50%)	Stigmasterol(**82**) (G = 14%)	5% acetone in water (GI = 0%)	Round up® (GI = 97%)

Figure 16. Bioassay result of compounds isolated from *A. aspera*

2.3.11. Isolation of compounds from *Premna schimperi*

The hexane, chloroform and methanol soluble portions of the 20% EtOH extract of the leave of *Primena schimperi* were assayed for its phytotoxic activity against *Parthenium hysterophorus* weed. The nonpolar fraction exhibited extract displayed better activity and hence 100 g leave of *P. schimperi* was extracted directly with $CHCl_3$ to afford 6 g (6%) crude extract. This was then applied over silica gel cholomn chromatography and resulted in the isolation of three compounds identified using different spectroscopic techniques as kaempferide, lebdane diterpene and 1-heptacosanol.

2.3.12. Characterization of compounds isolated from *P. schimperi*

Compound **99**

Compound **99** was isolated from fraction 10. Fraction ten (80 mg) was applied on PTLC and compound **99** (20 mg) was obtained as a jelly pure material. The TLC profile of this compound developed using hexane:EtOAc (3:2) solvent system and vanillin in sulfuric acid as a spraying agent showed a single pink spot (Rf 0.54).

Figure 17. TLC profile of compound **99**

The UV-Vis spectrum of compound **99** showed no absorption bands in the UV-Visible region. It is, therefore, suggestive to disclose the absence of any conjugated chromophore in the compound.

The IR spectrum of compound **99** showed a broadband at 3429 cm^{-1} indicative for the presence of O-H stretching. The methyl C-H stretching is displayed at 2924 cm^{-1}. The sharp peak displayed at 1632 cm^{-1} is evident for the presence of carbonyl functional group in the molecule. IR band at 1384 cm^{-1} is directive of the presence of geminal dimethyl stretching.

In the ^{1}H-NMR spectrum of compound **99** (Appendix 17), the presence of exocyclic protons is confirmed by the downfield signals appeared at *δ* 6.19 (1H, *s*) and *δ* 5.92 (1H, *s*). The HH-COSY experiment showed the correlation of these exocyclic protons with isolated methylene protons appeared at *δ* 3.30 (2H, *s,* H-14). The other downfield signal observed at *δ* 5.15 (1H, *m*) is evident for the presence of an olefinic methine proton which is correlated with protons appeared at *δ* 1.57 (3H, *s*, H-18) and 1.96 (2H, *m*, H-2). Two doublets of doublet signals showed at *δ* 2.65 (1H, *d*, *J* = 15.6) and δ 2.85 (1H, *d*, *J* = 15.2) are assigned from two diastereotopic protons (H-11). The ^{1}H-NMR experiment of compound **99** disclosed the presence of four terminal methyl protons each appeared at *δ* 1.57 (3H, *s*), *δ* 0.99 (3H, *s*), *δ* 0.86 (3H, *d*, *J* = 6.8 Hz) and *δ* 0.82 (3H, *s*).

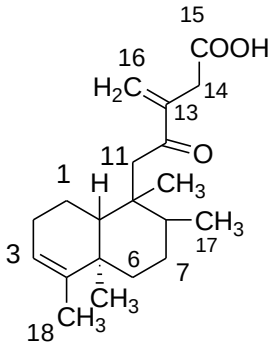
The ^{13}C-NMR experiment with the aid of DEPT-135 revealed the presence of six quaternary, three methine, seven methylene and four methyl carbons. The most downfield signal observed at δ 201.2 is assigned for α, β-unsaturated ketone carbon. The ^{13}C-NMR spectrum with the help of HMBC experiment confirmed presence of α, β-unsaturated acid functional group appeared at *δ* 176.1. The HMBC experiment showed correlation with an isolated methylene proton appeared at *δ* 3.3. The ^{13}C spectrum of compound **99** exhibited four olefinic carbons appeared at *δ* 143.8, *δ* 143.3, *δ* 126.8 and *δ* 120.5. Carbon signals at *δ*143.8 and *δ* 126.8 are due to exocyclic double bond in consistence with proton spectrum.

Table 20. ^{1}H (400 MHz) and 13C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **99** literature values of clerodane diterpene (360 MHz), $CDCl_3$)

Experimental data of compound **99**			Literature data of clerodane diterpene [99]	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	19.6		19.7	
2	36.1		36.3	
3	120.5	5.15 (1H, *s*)	120.6	5.1 (1H, *s*)
4	143.3		143.4	
5	38.3		38.5	
6	27.4		27.6	
7	26.5		26.6	

8	37.1	1.96 (1H, *m*)	37.0	1.88 (1H, *m*)
9	42.1		42.3	
10	46.6	1.68 (1H, *dd*)	46.8	1.68 (1H, *dd, J* = 12.1)
11	43.3	2.85, 2.61 (2H, *dd*, *J*=15.2)	43.5	2.88, 2.59 (2H, *dd*, *J*=15.2Hz)
12	201.3		201.2	
13	143.8		143.8	
14	36.1	3.30 (2H, *s*)	36.3	3.27 (2H, *s*)
15	176.1		176.1	
16	126.6	5.92 (1H,*s*), 6.19 (1H,*s*)	126.6	5.87 (1H, *s*), 6.14 (1H, *s*)
17	17.6	0.86 (3H, *d, J=6.8 Hz*)	17.6	0.83 (3H, *d*, *J* = *6.8Hz*)
18	19.9	1.57 (3H, *s*)	20.0	1.53 (3H, *s*)
19	17.9	0.96 (3H, *s*)	18.0	0.96 (3H, *s*)
20	16.6	0.82 (3H, s)	16.6	0.78 (3H, s)

The above data for compound **99** were compared with literature values of the clerodane diterpene previously isolated from *P. schimperi* and *P. oligotricha* and found to be in close agreement [100, 101, 135]. This compound was reported to exhibit strong antibacterial and antileishmanial activities [136].



99

Compound **112**

Compound **112** (30 mg) was obtained as a yellowish crystal from fraction nine and ten. The TLC profile of compound **112** showed a yellow spot in hexane:EtOAc (3:2) solvent system with an Rf value of 0.51 when it sprayed with vanillin in sulfuric (1%).This compound melts at 219-220^{o}C (lit. 227-231^{o}C) [137].

Figure 18. TLC profile of compound **112**

The UV-Vis spectral analysis indicated a characteristic of flavonoid chromophore with λmax at 268 and 364 nm. The IR spectrum of compound 100 showed three bands at 3301, 3455 and 3515 cm^{-1} each assigned for O-H stretching of a hydroxyl group of a flavonoid found in the different environment. The bands appeared at 1667, 1512 and 1162 cm^{-1} are interpreted for the presence of carbonyl group, aromatic group C=C stretching and C-O stretching respectively.

The ^{1}H-NMR spectrum of compound **112** (Appendix 18) displayed clearly well-resolved signals in the aromatic region. The most downfield signal appeared at *δ* 12.44 (1H, *s*) is a result of strong hydrogen bonding between hydroxyl group at C-5 and a carbonyl group at C-4. The other downfield signals at *δ* 10.83 (1H *br s*) and *δ* 9.47 (1H, *br s*) are indicative for the presence of two more hydroxyl groups in the compound. The doublet signals appeared at *δ* 8.12 (2H, *d*, *J*= 8.8 Hz), *δ* 7.09 (2H, *d*, *J* = 8.8 Hz) are suggestive for the presence of symmetrical aromatic *ortho* coupled protons (H-2'/6' and 3'/5'). These doublet signals are also indicative the fourth position is substituted by a certain group; most probably the methoxy group appeared at *δ* 3.83 (3H, *s*). The ^{1}H NMR spectrum of

compound **112** also showed the presence two additional aromatic protons, *meta*-coupled doublet at *δ* 6.45 (1H, *d*, *J* = 2 Hz, H-8) and *δ* 6.19 (1H, *d*, *J* = 2 Hz, H-6). The presence of these all aromatic protons (two *ortho* coupled and two *meta* coupled) are suggestive the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl rings. The later ring was further confirmed to be *p*-hydroxyphenyl system from the ^{13}C-chemical shift of the carbon signals at *δ* 129.7 (C-2', 6') and *δ* 114.4 (C-3', 5').

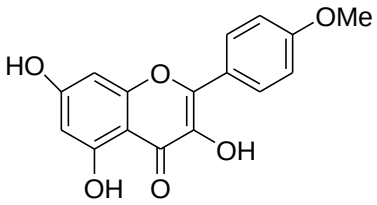
The ^{13}C-NMR data together with DRPT-135 displayed the presence of nine quaternary, four methine and one methyl carbons. The most downfield (at *δ* 176.4) and the most upfield (at *δ* 55.8) signals are assigned for α, β-unsaturated carbonyl carbon the flavonol and methoxy carbon of compound 100 respectively. The methine carbons at *δ* 129.7 (C-2', C-6') and *δ* 114.5 (C-3', C-5') were due to symmetrically placed aromatic carbons on asymmetrically *para* substituted B-ring of the flavonoid. The compound exhibited oxygenated aromatic quaternary carbons at *δ* 146.4 (C-2), *δ* 136.5 (C-3), *δ* 161.1 (C-5), *δ* 164.4 (C-7), *δ* 159.6 (C-9) and *δ* 160.9 (C-4'). The additional quaternary carbons observed were at *δ* 123.7 and 103.5 due to C-1'and C-10 respectively. All physical and spectroscopic data were compared with literature reports of kaempferide and was found in a good agreement [137].

Table 21. ^{1}H (400 MHz) and 13C-NMR (100 MHz) spectral data ($CDCl_3$) of compound **112** with literature values of kaempferide

Experimental data of compound **112**			Literature data of Kaempferide [137]	
	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
2	146.4		146.3	
3	136.5		136.1	
4	176.4		176.1	
5	161.1		160.8	
6	98.7	6.20 (1H, *s*)	98.3	6.18 (1H, *d*)
7	164.4		164.3	
8	93.9	6.45 (1H, *s*)	93.6	6.37 (1H, *d*)
9	156.6		156.3	
10	103.5		103.7	

1’	123.7		123.3	
2’	129.7	8.12 (1H, *d, J=8.8*)	129.4	8.12 (1H, *d*)
3’	114.4	7.09 (1H, *d*)	114.1	7.09 (1H, *d*)
4’	160.9		160.6	
5’	114.4	7.09 (1H, *d*)	114.1	7.09 (1H, *d*)
6’	129.7	8.14 (1H, *d*)	129.4	8.12 (1H, *d*)
O-CH_3	55.8	3.83 (3H, *s*)	55.4	3.83 (3H, *s*)
3-OH		9.47 (1H, *s*)		9.45 (1H, *s*)
5-OH		12.45 (1H, *s*)		12.32 (1H, *s*)
7-OH		10.83 (1H, *s*)		10.57 (1H, *s*)

Based on all physical and spectroscopic data the compound was proposed to be kaempferide. This compound was previously isolated from *Premna schimperi* [137]. The proposed structure is depicted below



112

Compound **118**

In the investigation of secondary metabolites from *P. schimperi* and other biologically active plants, compound **118** was isolated from fraction 5 as a white powder (29 mg) melting at 75-76^{o}C. The TLC developed using mobile phase hexane: EtOAc (4:1) was visualized as a pink spot (Rf 0.5) after spraying with vanillin in sulfuric acid. The UV-Vis spectrum (in $CHCl_3$) showed absorption neither in the UV nor in the visible region. The IR spectrum displayed signals at 3395 cm^{-1} (for O-H stretching), 2924 and 1470 cm^{-1} (for C-H stretching and bending) and 708 for C-C stretching. This compound is also isolated from *Warburgia ugandensis* bark in this work and all physical and spectroscopic parameters are found similar.

2.3.13. Bioassay result of compounds isolated from *P. schimperi*

The *in vitro* result of $CHCl_3$ extracts of *P. schimperi* showed 78% growth inhibition at 0.05 mg/mL concentration. Following the *in vitro* result, the $CHCl_3$ extract was applied on column chromatography to afford three compounds. These are kaempferide, diterpene, heptacosanol. All the three compounds showed 100% seed germination inhibition *in vitro*.

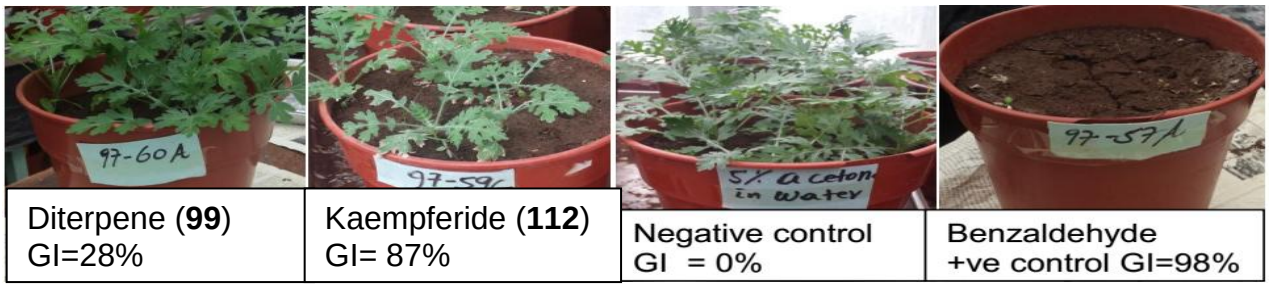
Table 22. *In vitro* phytotoxicity results of compounds isolated from *P. schimperi*

compound	source	number of germinated seeds					
		T1	T2	T3	mean	STDV	%GI
112	*P. s*	0	0	0	0	0	100
118		0	0	0	0	0	100
99		0	0	0	0	0	100
Roundup®		0	0	0	0	0	100
5% acetone		8	7	9	8	1	0

In vivo bioassay results displayed the only kaempferide inhibit the growth of the 15-day seedling with 87%. The diterpene compound **99** and heptacosanol (**118**) showed minimum growth inhibition with 29 and 42% respectively. Even though the diterpene compound **99** reported to have strong antibacterial activity, its phytotoxic activity is insignificant.

Table 23. *In vivo* result of pure compounds from *P. schimperi*

compounds	ADB of seedlings before treatment (mg)				ADB of seedling after treatment (mg)				Mass d/nce	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
112	96	100	104	100	540	532	488	520	420	87
118	96	100	104	100	1990	1995	2015	2000	1900	42
99	96	100	104	100	2455	2420	2475	2450	2350	29
Roundup®	96	100	104	100	140	141	139	140	40	98
5% acetone	96	100	104	100	3400	3444	3436	3426.7	3326	0



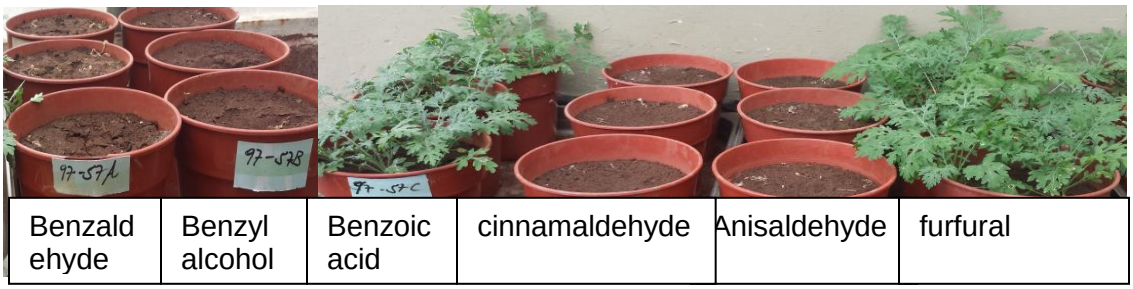
Diterpene (**99**) GI=28%	Kaempferide (**112**) GI= 87%	Negative control GI = 0%	Benzaldehyde +ve control GI=98%

Figure 19. *In vivo* results of compound **99** and **112** in comparison with the standard

2.3.14. Phytotoxicity study of synthetic chemicals

To study the structural activity relationship (SAR) of aldehydic compounds isolated in the course, and benzaldehyde (**125**) and its oxidative and reductive derivatives colleceted from our laboratory were screened for their phytotoxicity activity against the invasive *P. hysterophorus* weed at 1 mg/mL concentration *in vivo*. The result displayed that benzaldehyde (**125**), anisaldehyde (**126**), benzyl alcohol (**127**), cinnamaldehyde (**128**) and 3-pyridine carboxaldehyde (**129**) exhibited strong activity (90%) in suppressing Parthenium seedling growth. The other compounds like benzoic acid (**130**), furfural (**131**), *p*-Me_2N- benzaldyhide (**132**), furfurol (**133**), and *p*-cresol (**134**) exhibited insignificant activity against the growth of *P. hysterophorus* weed.

Previous study on the structural activity relation (SAR) suggested that benzaldehyde (**125**) is a new class candidate for plant growth regulator on *Brassica campestris* [138]. However, in another study, thirty-seven commercial aldehydes containing aliphatic chains and aromatic rings as well as heteroaromatic rings were evaluated for their inhibitory activities against Chinese amaranth (*Amaranthus tricolor* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) and the result displayed that the activity mainly depends on unsaturation of structures, type, number and position of substituents and concentrations of aldehydes [139]. The result observed in our experiment also indicate that the activity mainly depends on the type of the compounds used.



Benzaldehyde	Benzyl alcohol	Benzoic acid	cinnamaldehyde	Anisaldehyde	furfural

p-Me_2N- BA	3-pyridine carboxaldehyde	furfurol	*p*-cresol	5% acetone in H_2O (-ve control)	folmaldehyde

Figure 20. Bioassay results of small molecules

3. Conclusion

Nontoxic, easily biodegradable and hence environmentally friendly herbicides from the natural origin are needed in organic agriculture. In this study, more than 50 plants were evaluated for their phytotoxic activity against *Parthenium hysterophorus* weed. 20% of the screened plants showed good phytotoxic activity by suppressing the seed germination over 80% GI under laboratory condition. The *in vitro* result showed that $CHCl_3$ soluble portion of the ethanol extracts of *E. kebericho, W. ugandensis, A. aspera, S. gigas, Z. scabra, A. indica* and *E. globulus* inhibited the seed germination by 100% at 0.05% concentration. The herbicidal potential of these plants were also studied by treating on 15 days old seedlings under greenhouse condition and only the $CHCl_3$ extracts of *E. kebericho, W. ugandensis, S. gigas, P. schimperi* and *A. aspera* were found to be effective in suppressing the growth of the seedling by 92, 94, 85, 78 and 76% respectively.

Dehydrocostus lactone (**28**) from *E. kebericho* root, muzigadial (**48**) and cinnamolide-3β-acetate (**64**) from *W. ugandensis* bark and stigmasterol glycoside (**121**) from *A. aspera* aerial part and kaempferide (**112**) from *P. schimperi* leaf were found to be active principles. Dehydrocostus lactone (**28**), muzigadial (**48**) and cinnamolide-3β-acetate (**64**) showed the highest herbicidal activity against *P. hysterophorus* weed with 92, 95 and 91% growth inhibition respectively. The activity exhibited by these compounds is almost similar to the phytotoxic effect of the positive control commercial herbicide Roundup®. The post-emergent assay for stigmasterol glycoside (**121**) showed that it inhibited the seedling growth by 50% only. Therefore, extracts containing dehydrocostus lactone (**28**), muzigadial (**48**) and cinnamolide-3β-acetate (**64**) may serve as natural herbicides in the management of *P. hysterophorus* weed.

4. Experimental part

4.1. Plant collection

A total of 50 different plants were collected from different geographical areas and evaluated for their herbicidal activities on *P. hysterophorus* weed. Some of them were obtained from Ambo APPRC by Shashitu based on their literature information, others from Wef washa, Yayu, Arsi and Addis Ababa region by different individuals.

4.2. Materials used for purification and spectroscopic analysis

The compounds reported in this work were isolated using two sizes of column chromatography on silica gel and aluminum oxide (neutral), a medium size and small size which can carry 80 g and 12 g silica gel respectively. Fractions collected from CC were purified by sephadex LH-20. Preparative thin layer chromatography was run on 1.0mm thick layer of silica gel. The silica gel used for the CC is 60-120 mesh particle size. TLC was performed on precoated plates (Silica gel 60 F_{254}, 230-400 mesh, Merck) and aluminum oxide plates; Melting points are uncorrected; detection by UV light at 254 and 366 nm and spray reagents vanillin-H_2SO_4; IR: KBr disk or neat and measured on a Perkin Elmer 1600 and Pye Unicam Infrared spectrophotometer SP3-300. UV spectra were measured on a Shimadzu UV-VIS recording spectrophotometer, UV-160, spectronic genesys spectrophotometer; ^1H and ^{13}C NMR were recorded, in $CDCl_3$, DMSO, $(CD_3)_2CO$ and CD_3OD using the solvent peak as reference (chloroform: δ_H 7.2 and δ_C 77.2, DMSO, δ_H 2.5 and δ_C 39.5, deuterated acetone: δ_H 2.05 and δ_C 29.5 and 205.5 methanol: δ_H 3.3 and δ_C 49.0). Chemical shift values were reported in δ (ppm) units, the solvent signals as internal references; ^1H, ^{13}C, and 2D-NMR spectra were obtained on Jeol F X 90 Ω spectrophotometer at 90 and 22.5 MHz; Jeol JNM-EX400 instrument at 400 MHz and 100 MHz; a Bruker Ultrashield^{TM} 400 spectrometer at 400 and 100 MHz with TMS and solvents as internal standard and δ values are given in ppm relative to TMS internal standard. EIMS was obtained on a Finnigan MAT 95Q and VG Quattro quadrupole mass spectrometer (70 eV).

4.3. Extraction of plants for bioassay test

Plant materials were collected, air dried and finely ground. Each plant material (20 g) was extracted with ethanol by putting on the shaker for 24 h. The EtOH extract was concentrated with rotary vapor under reduced pressure at 40^{o}C and 100 mg were weighed from each and diluted in 5% acetone in water solvent system (100 mL) to prepare 1 mg/mL concentrated test solution.

4.4. Collection and preparation of *P. hysterophorus* weed seeds for bioassay

The seeds of *Parthenium hysterophorus* were air-dried, threshed by hand, and intact and viable seeds were selected. The selected seeds were surface sterilized by shaking them for five minutes in 1% Sodium hypochlorite (NaOCl) solution and washed with distilled water for three minutes immediately before use to avoid any fungus that would be developed during the screening test.

4.5. Preliminary phytotoxicity screening test procedure (*in vitro*)

The phytotoxicity was conducted at Ambo Plant Protection Research Center (APPRC), Ethiopia Institute of Agricultural Research (EIAR), which is 115 km from Addis Ababa, 8° 58' 36.5" latitude and 37° 50' 40.6" longitude. The temperature of APPRC is Max: 26°C Min: 11°C, Rainfall (mm):1100 annual. Major soil types: Vertisols consisting of 67% clay, 18% silt, 15% sand and 1.5% organic matter (http://www.eiar.gov.et)

Ten pre-washed and disinfected test seeds were sowed on each Petri-dish linked with filter paper. Each plant extract (100 mg) was diluted in 5 mL of acetone and then water (95 mL) was added to make the solution 1mg/mL. The final concentration of acetone in water is 5%. The germination inhibition effect of the solvent system was studied as a negative control. Each test solution was watered every day until the filter paper moisturized for 15 successive days. The experiment was done in triplicate for each extract using Completely Randomized Design. 5% acetone in water was used as a negative control whereas a standard Commercial herbicide (Roundup®) used as a positive control. The seed germination response is the main detrimental factor for the screening of botanicals for their herbicidal activity. After fifteen days the number of germinated seeds was counted. The presence of growth-altering secondary metabolites in the test botanicals was observed by looking at the seed germination response.

Treatment and control assays were performed in triplicate. Petri dish plates were covered and placed in the greenhouse at the temperature 23-30^{o}C. Germination inhibition of extracts and pure compounds is calculated using a standard formula GI% = [1-(Gt/Gc)] × 10% [140], where Gt and Gc are the numbers of germinated seeds in treatment and control respectively.

4.6. *In vivo* test procedures

The *in vivo* bioassay tests were done in the greenhouse with the standard protocol [141]. Three soil types (red soil, sand, and compost) were collected from Guder 15 km far from Ambo and APPRC garden. The soil was mixed with a ratio of 2:1:1 to the red soil, sand and compost. The mixed soil was sterilized at Ambo University. Sufficient equal size plastic pots of the upper diameter 20 cm and 18 cm deep were prepared. Each pot was filled with 3 kg mixtures of soil. Each pot was watered until it moisturized and placed in a greenhouse at the temperature of 23-30^{o}C. Initially, 10 viable parthenium seeds were sowed in each pot and watered every day for 15 days. After 15 days, germinated seedlings were thinned to 3 uniform seedlings for the treatment application. The plant extract test solutions were sprayed using foliar spray bioassay technique for 15 days in an average of 5 mL per spray (2 drops per leaf) [142, 143]. Treatments were arranged using Completely Randomized Block Design with three replications for each test solution. The solvent (5% acetone in water) and roundup were used as a negative and positive control respectively. After two weeks of treatment, seedlings of the control and treatment were carefully uprooted and their roots were washed with water and allowed to dry separately [142, 143]. The dry biomass of the seedlings before treatment and after treatment was recorded and the percentage growth inhibition was calculated using the standard formula [109].

$$GI\% = \left[1- \frac{\text{average dry biomass difference of treatment}}{\text{average dry biomass difference of control}} \right] \times 100\%$$

The minimum inhibition concentration was determined by taking 2 mL, 5 mL, 10 mL and 20 mL from the stock solution (1 mg/mL) and diluted at 100 mL of water to prepare 0.02, 0.05, 0.1, 0.2 and 1 mg/mL test solution.

4.7. General extraction method

Extraction of plant samples was conducted using cold solvent-solvent extraction procedures. Each plant sample study was dried and ground finely with a grinder. Powder of each sample (20 g) was soaked in separate 300 mL Erlenmeyer flasks containing solvents of 100 mL ethanol with occasional shaking. Based on the biological activity test results, plant extracts which showed good activity was partitioned with solvents of different polarity (hexane, $CHCl_3$, and MeOH), filtered and concentrated. Finally, the resulting crude extracts were subjected to bioassay for their phytotoxicity activities against *P. hysterophorus* weed.

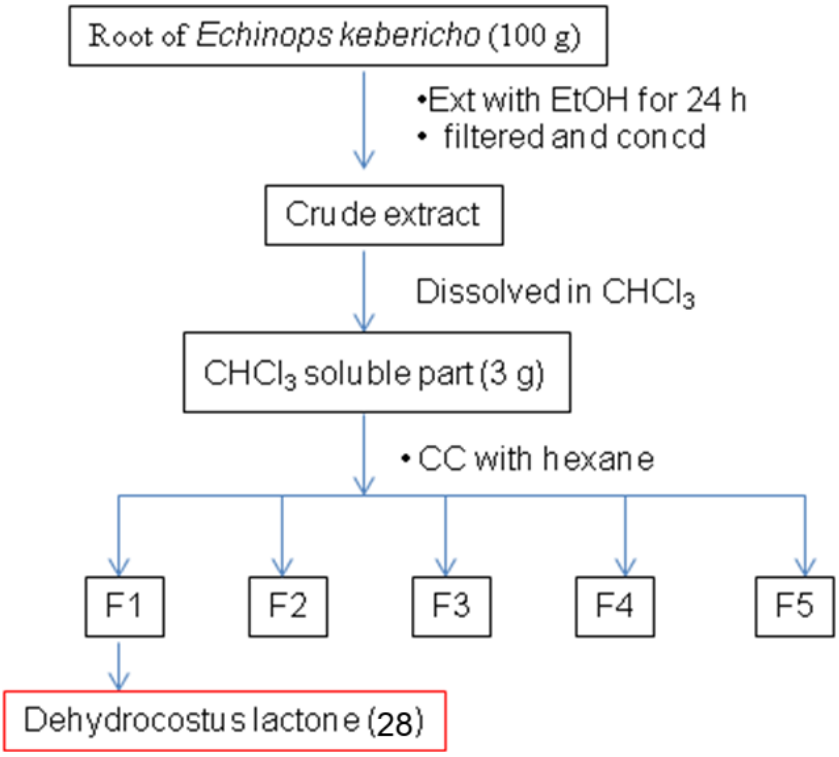
Plants exhibited highest activity were identified and extracted in large scale (100 g each) with EtOH (500 mL) for 24 h on the shaker. The extract was filtered and concentrated with a rotary evaporator at 30-40^oC. It was then allowed to be dissolved in $CHCl_3$ and the $CHCl_3$ soluble part was filtered and concentrated using a rotary evaporator under reduced pressure. The crude $CHCl_3$ extract of each plant was packed with silica gel and applied on column chromatography. The fractions were collected and combined based on their TLC profile. Each combined fraction was concentrated and then subjected to its biological activity and the pure herbicidal compounds were isolated from the active fraction. Purification of isolated compounds was done using column chromatography, Sephadex LH 20, PTLC, and recrystallization techniques.

The NMR spectral data were performed with instruments Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr. UV data were recorded using T60 UV-VS spectrophotometer.

4.7.1. Extraction and isolation for the chemical study of *E. kebericho* root

Root of *E. kebericho* was purchased from Merkato market, Addis Ababa, Ethiopia. The roots of this plant were ground in to a fine powder with grinder. The ground root of *E. kebericho* (100 g) was extracted with EtOH (500 mL) on shaker for 6 h, filtered and concentrated with rotary evaporator at 40^oC to afford 12 g (12%) semi-solid crude extract. It was then washed with $CHCl_3$ and the $CHCl_3$ extract (3 g) was adsorbed on silica gel

and applied to silica gel (70 g) column chromatography. The column was eluted with hexane, hexane:EtOAc of increasing polarity and 12 fractions were collected and combined to be reduced into five subfractions based on their TLC profile. Compound **28** (480 mg) was obtained as a white crystal from subfraction 1.



Dehydrocostus lactone (480 mg): white solid; soluble in $CHCl_{3;}$, mp 55-56^{o}C; Rf 0.5 (mobile phase hexane:EtOAc, 4:1); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 6.12 (1H, *d*, *J* = 3.6 Hz, H-13), δ 5.43 (1H, *d*, *J* = 2.8 Hz, H-13), δ 5.19 (1H, *s,* H-15), δ 4.99 (1H, *s,* H-15), δ 4.83 (1H, *s,* H-14), δ 4.74 (1H, *s,* H-14), δ 3.90 (1H, *t*, *J*= 9.4 Hz, H-6), δ 2.85 (2H, *m,* H-1, H-7), δ 1.87 (2H, *m*), δ 2.47 (2H, *m*), δ 2.20 (1H, *m*), 2.10 1H, *m*), δ 2.44(1H, *m*), δ 1.37 (1H, *m*); ^{13}C NMR (100 MHz, CDC_3): 47.47 (C-1), 30.27 (C-2), 32.62 (C-3), 151.37 (C-4), 45.03 (C-5), 85.30 (C-6), 51.95 (C-7), 30.99 (C-8), 36.3 (C-9), 149.24 (C-10), 139.69 (C-11), 170.19 (C-12),120.18 (C-13), 109.53 (H-14) and 112.50 (C-15).

4.7.2. Extraction and isolation of compounds from *W. ugandensis* bark

Warburgia ugandensis bark and leaves were collected from Bale Robe, Oromia region, south-central Ethiopia which has latitude of 6° 44' 59.99" N and longitude of 40° 14' 60.00" E. It is the area found at an altitude of 2,492 metres and 430 kilometres far away by road from Ethiopia's capital Addis Ababa. The specimen of the plant was deposited at the National Herbarium Department of Biology; Addis Ababa University Herbarium with voucher number of 97-41A and its identity was determined by a plant taxonomist.

The ground bark of *W. ugandensis* (150 g) was extracted with ethanol (500 mL) on shaker for 24 h, filtered and concentrated using rotary evaporator at 40^{o}C to afford 15 g red like crude extract. This was then partitioned with EtOAc to afford 10 g EtOAc soluble part. The EtOAc soluble part (4 g) was adsorbed on silica gel and subjected to silica gel (80 g) column chromatography. The column was eluted with hexane:EtOAc of increasing polarities to afford 12 combined fractions collected based on their TLC profile.

Combined fraction 2 (80 mg) (hexane 100%) was applied on PTLC using hexane:EtOAc (9:1) as eluent solvent. The major band observed was collected, dissolved in $CHCl_3$ and filtered using ground filtration. This band showed UV active (254 nm) single TLC spot in hexane:EtOAc (4:1) solvent system. It was finally identified to be mixtures of polygodial and 9-deoxy muzigadial (20 mg).

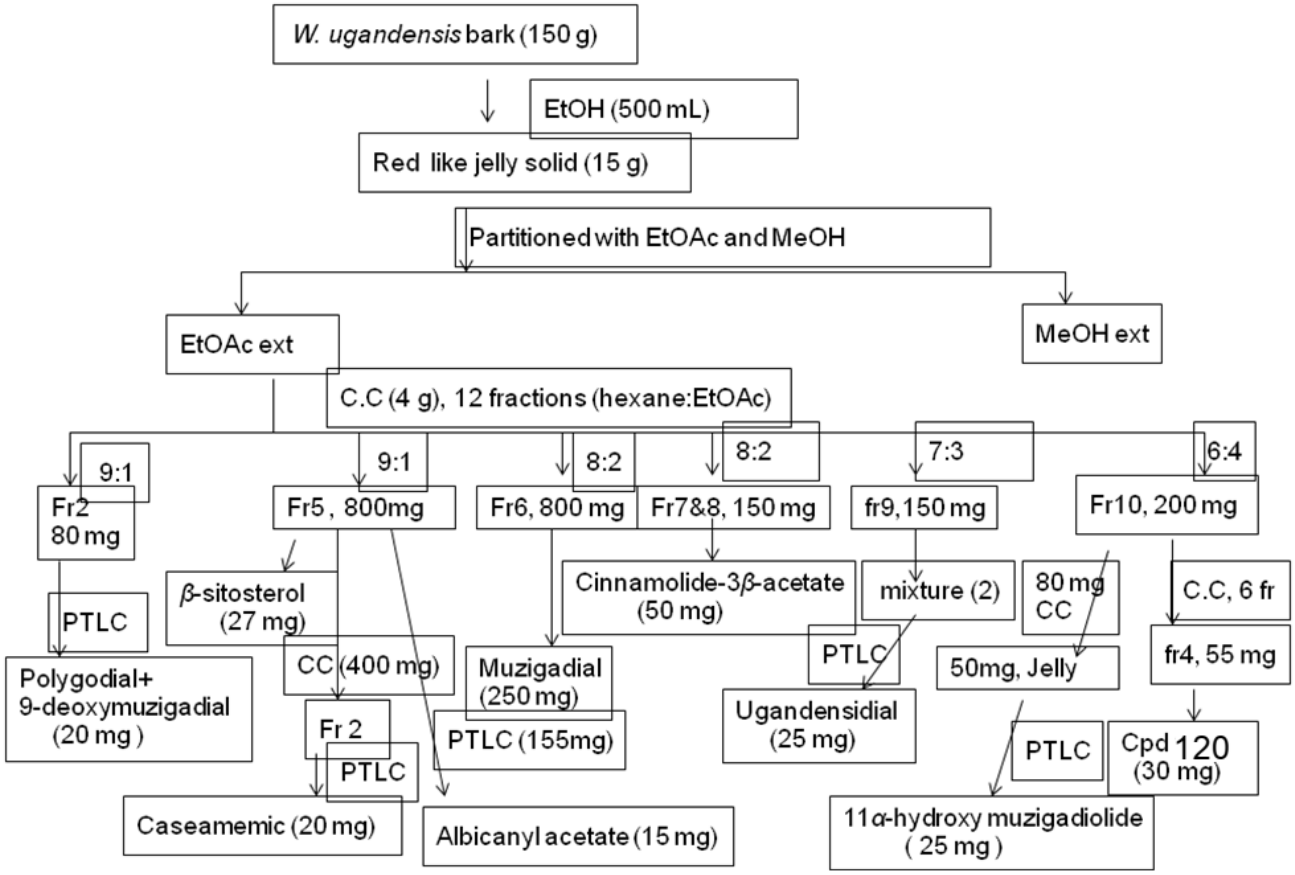
From combined fraction 5 (800 mg), white powder was precipitated on the surface of the vial from hexane:EtOAc (9:1) solvent system and identified to be *β*-sitosterol (27 mg). From fraction 5, 155 mg was taken and applied on PTLC and compound 103 was isolated as a pure compound and identified as albicanyl acetate (15 mg). from the remaining part of fraction 5 (400 mg) was applied on silica gel (10 g) column chromatography and eight subfractions were collected. Subfraction 2 (60 mg) was further purified by PTLC and identified to be Caseamemin (20 mg).

Fraction 6 (800 mg) allowed to stay overnight on the vial and a white crystal was formed from hexane:EtOAc (4:1) solvent system and identified to be muzigadial (250 mg).

Fraction 7 & 8 (300 mg) formed a white powder on the walls of the vial using hexane:EtOAc (4:1) solvent system and found to be Cinnamolide-3β-acetate (50 mg).

Fraction 9 (150 mg) obtained from hexane:EtOAc (7:3) solvent system. A white crystal was precipitated which was found in mixtures of two compounds. The two mixture compounds were separated into their pure form by using preparative thin layer chromatography (PTLC) using hexane:EtOAc (7:3) solvent system. Two bands were observed, each band collected separately, dissolved in $CHCl_3$ and filtered with ground filtration technique and identified to be ugandensidial (10 mg) and cinnamolide 3β-acetate.

Fraction 10 (200 mg) was adsorbed on silica gel and applied on silica gel (12 g) column chromatography. It was eluted with hexane:EtOAc solvent system of increasing polarity. Seven subfractions were collected. From subfraction 4, compound **120** was obtained (30 mg). Fraction 10 (80 mg) was applied on Sephadex LH20 using $CHCl_3$:MeOH (1:1). Two fractions were collected. The first fraction gave oil like jelly material found to be 1-heptacosanol (10 mg). The second fraction (50 mg) was crystallized with hexane to afford a white solid material identified to be 11α-hydroxy muzigadiolide (25 mg).



Hentriacontane (60 mg) was obtained from EtOAc extracts of *W. ugandensis* leaves. The crude extract (4 g) was adsorbed on silica gel and applied on silica gel (4 g) column chromatography. Six combined fractions (based on their TLC profile) were collected and a white amorphous solid material was precipitated from the first fraction and proposed to be nonacosane (60 mg).

Muzigadial (250 mg): white crystal; soluble in $CHCl_3$; mp 120-122^oC; Rf 0.42 (mobile phase hexane: CHCl3:EtOAc ,1:1:1); UV (EtOH) λmax nm: 224; IR υ_{cm-1}: 3462 (OH), C-H (2957), 1720 and 1663 (*α, β* unsaturated C=O),1638 and 900 (exocyclic methylene); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant *J* in Hz: *δ* 1.03 (1H, *m,* H-1), 1.00 (1H, *ddd*, *J* = 4, 4, 4.8 Hz, H'-1), 1.13 (1H, td, *J*= 13, 4, H-2), 1.69 (1H, *m,* H'-2), 1.71 (1H, *m*, H-3), 2.61 (1H, *m*, H-5), 2.51 (2H, *m*, H-6), 7.23 (1H, *t, J* = 3.4 Hz, H-7), 4.07 (1H, *d, J* = 1.2 Hz, H-9), 9.63 (1H, *s,* H-11), 9.44 (1H, *s,* H-12), 4.93 (1H, *s,* H_a-13) 4.77 (1H, *s,* H_b-13), 1.07 (3H, *d, J* = 6.4 Hz) and 0.86 (3H, *s*); ^{13}C NMR (100 MHz, CDC_3): δ 31.86 (C-1), 31.73 (C-2), 38.27 (C-3), 151.61 (C-4), 40.22 (C-5), 27.64 (C-6), 155.83 (C-7), 139.89 (C-8), 77.61 (C-9), 42.35 (C-10), 201.33 (C-11), 192.77 (C-12), 106.14 (C-13),18.47 (C-14) and 15.11 (C-15).

Cinnamolide-3β-acetate (50 mg): white powder; soluble in $CHCl_3$; mp 141-142^oC; Rf 0.42 (mobile phase hexane:EtOAc,3:2); UV (EtOH) λmax nm: 224; IR υ_{cm-1}: 1763 and 1680 (*α, β* unsaturated C=O), 1730 (acetate group);^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant *J* in Hz: *δ* 1.42 (1H, *m,* H-1), 1.64 (1H, *m,* H'-1), 1.69 (1H, *m,* H-2), 1.74 (1H, *ddd*, *J* = 8, 4.4 Hz, H'-2), 4.55 (1H, *dd, J* = 11, 4.4 Hz, H-3), 1.47 (1H, *dd, J* = 11, 5.4 Hz, H-5), 2.22 (1H, *m*, H-6), 2.45 (1H, *dq*, *J* = 20, 4 Hz, H'-6), 6.90 (1H, *dd, J* = 3.2 and 3.6 Hz, H-7), 2.83 (1H, *m*), 4.06 (1H, *t*, *J* = 9 Hz, H_a-11), 4.41 (1H, *t*, *J* = 9.2 Hz, H_b-11), 0.94 (3H, *s,* H-13) and 2.09 (3H, *s,* methyl protons of acetate group); ^{13}C NMR (100 MHz, CDC_3): δc 36.85 (C-1), 23.50 (C-2), 80.12 (C-3), 37.61 (C-4), 49.25 (C-5), 24.63 (C-6), 135.88 (C-7), 127.14 (C-8), 50.54 (C-9), 33.99 (C-10), 66.97 (C-11), 169.86 (C-12), 27.79 (C-13), 15.98 (C-14), 13.49 (C-15), 21.25 (methyl carbon of the acetate unit) and 170.84 (Carbonyl carbon of the acetate).

Ugandensidial or cinnamodial (10 mg): white needle like crystal; soluble in $CHCl_3$; mp 134-135^oC; Rf 0.48 (mobile phase hexane:EtOAc,4:1); UV (EtOH) λmax nm: 223.4; IR

υ_{cm-1}: 3453 (OH stretching), 1736 and 1680 (*α, β* unsaturated C=O stretching), 1736 (acetate group), 1370 (geminal dimethyl stretching), 1230 and 1024 (C-O stretching and bending); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant *J* in Hz: δ_H 5.91 (1H, *t*, *J* = 4.8 Hz, H-6), 7.02 (1H, *d, J* = 5.6 Hz, H-7), 4.03 (1H, *br s,* OH-9), 9.78 (1H, *s,* H-11), 9.50 (1H, *s,* H-12), 1.34 (1H, *s*, H-13), 1.17 (3H, *s*, H-14), 1.01 (3H, *s*, C-15), 2.14 (3H, *s,* methyl proton of acetate group); ^{13}C NMR (100 MHz, CDC_3): δ_C 31.79 (C-1), 19.95 (C-2), 43.97 (C-3), 34.00 (C-4), 44.91 (C-5), 66.03 (C-6), 148.73 (C-7), 140.88 (C-8), 77.3 (C-9), 41.63 (C-10), 201.2 (C-11), 193.11 (C-12), 32.58 (C-13), 21.49 (C-14), 17. 66 (C-15), 24.76 (methyl carbon of the acetate unit) and 170.09 (C=O of acetate group).

β-sitosterol (27 mg): white solid; soluble in $CHCl_3$; mp 130-136^{o}C; Rf 0.62 (mobile phase hexane:EtOAc, 2:1); UV (EtOH) λmax nm: no absorbance; IR υ_{cm-1}: 3453 (OH stretching), 1736 and 1680 (*α, β* unsaturated C=O stretching), 1736 (acetate group), 1370 (geminal dimethyl stretching), 1230 and 1024 (C-O stretching and bending); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant *J* in Hz: δ_H 3.53 (1H, *m,* H-3), 5.37 (1H, *t, J* = 2 Hz, H-6), 0.93 (3H, *d, J* = 6.8 Hz, H-19), 0.84 (3H, *d, J* = 2 Hz, H-24), 0.84 (3H, *d, J* = 2 Hz, H-26), 0.82 (3H, *d,* H-27), 0.70 (3H, *s,* H-28) and 1.03 (3H, *s,* H-29); ^{13}C NMR (100 MHz, CDC_3): 37.26 (C-1), 31.68 (C-2), 71.84 (C-3), 42.32 (C-4), 140.76 (C-5), 121.75 (C-6), 31.92 (C-7), 31.98 (C-8), 50.14 (C-9), 36.53 (C-10), 21.11 (C-11), 39.78 (C-12), 42.34 (C-13), 56.78 (C-14), 26.05 (C-15), 28.28 (C-16), 56.05 (C-17), 36.17 (C-18), 19.43 C-19), 33.95 (C-20), 24.33 (C-21), 45.84 (C-22), 23.07 (C-23), 12.01 (C-24), 29.15 (C-25), 19.86 (C-26), 19.05 (C-27), 18.80 (C-28) and 11.89 (C-29).

11α-hydroxy muzigadiolide (25 mg): white solid; soluble in acetone or methanol; mp 181-182^{o}C; Rf 0.52 (mobile phase hexane: EtOAc, 4:1); UV (EtOH) λmax nm: 217; IR υ_{cm-1}: 3387 (OH), 2961(C-H), 1704 and 1630 (*α, β* unsaturated C=O stretching), 1629 and 884 (exocyclic double bond), 1171 (C-O); ^{1}H-NMR (400 MHz, acetone-d6), chemical shift *δ* in ppm, coupling constant in Hz: δ_H 1.43 (1H, *m*, H-1 eq), 2.37 (1H, *m*, H-1 ax), 1.72 (1H, *m*, H-2 eq), 1.20 (1H, m, H-2 ax), 2.19 (1H, *m,* H-3), 2.68 (1H, *m,* H-5), 2.3 (2H, *m,* H-6), 6.29 (1H, *dd, J* = 0.8, 1.2, H-7), 6.90 (1H, *s,* H-11), 5.68 (1H, br s, 11-OH), 4.80 (1H, *s,* H_a-13), 4.90 (1H, *s,* H_b-13), 1.08 (3H, d, *J* = 6.8 Hz, H-14) and 0.77 (3H, *s,* H-15); ^{13}C NMR (100 MHz, acetone-d6): δc 31.17 (C-1), 32.43 (C-2), 38.43 (C-3), 152.60 (C-4),

40.26 (C-5), 26.98 (C-6), 138.34 (C-7), 130.95 (C-8), 75.44 (C-9), 40.12 (C-10), 98.29 (C-11), 166.47 (C-12), 104.96 (C-13), 18.10 (C-14) and 14.58 (C-15).

Albicanyl acetate (15 mg): jelly material; soluble in $CHCl_3$; Rf 0.50 (mobile phase hexane: EtOAc, (5:1); no absorption in the UV-Vis region; IR υ_{cm-1}: 2923 (C-H stretching), 1735 (acetate unit), 1641 and 1461 cm-1 (exocyclic double bond stretching and bending), 1230 (C-O stretching), 1376 cm-1 (geminal dimethyl stretching); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant *J* in Hz: *δ* 1.25, 1.68 (2H, *m,* H-1), 1.45, 1.52 (2H, m, *qt,* H-2), 1.09 (1H, *m,* H-5), 2.06, 2.41 (2H, *m,* H-7), 2.09 (1H, *m,* H-9), 4.53 (1H, *s,* H_a-12), 4.87 (1H, *d,* H_b-12), 4.2 (1H, *dd, J*= 11.2, 9.2 Hz, H_a-11), 4.3 (1H, *dd, J*= 11.2, 3.6 *Hz,* H_b-11), 0.84 (3H, *s,* H-13), 0.77 (3H, *s,* H-14), 0.71 (3H, *s,* H-15) and 2.07 (3H, *s*, H-17); ^{13}C NMR (100 MHz, CDC_3): 39.03 (C-1), 19.17 (C-2), 41.92 (C-3), 33.94 (C-4), 55.06 (C-5), 23.90 (C-6), 37.60 (C-7), 146.83 (C-8), 54.73 (C-9), 38.97 (C-10), 107.15 (C-11), 61.58 (C-12), 33.64 (C-13), 21.76 (C-14), 15.11(C-15), 171.41 (C-16), 21.12 (C-17).

Polygodial (20 mg): obtained in the form of mixture with 9-deoxymuzigadial as jelly material ; soluble in $CHCl_3$; Rf 0.65 (mobile phase hexane: EtOAc, (4:1); IR υ_{cm-1}: 2924 (C-H stretching), 1726 and 1675 cm^{-1} (due to α, β unsaturated carbonyl group); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm: *δ* 1.37, 1.82 (2H, *m,* H-1), 1.48, 1.60 (2H, *m,* H-2), 1.19, 1.48 (2H, *m,* H-3), 1.25 (1H, *m,* H-5), 2.49, 2.32 (2H, *m,* H-6), 7.14 (1H, *m,* H-7), 2.83 (1H, *m,* H-9), 9.54 (1H, *ddd,* H-11), 9.50 (1H, *s,* H-12), 0.93 (3H, *s,* H-13), 0.95 (3H, *s,* H-14), 1.09 (3H, *d, J* = 6.8 Hz, H-15); ^{13}C NMR (100 MHz, CDC_3): 39.55 (C-1), 18.02 (C-2), 41.70 (C-3), 33.12 (C-4), 48.95 (C-5), 25.22 (C-6), 154.36 (C-7), 138.24 (C-8), 60.28 (C-9), 37.09 (C-10), 202.01(C-11), 193.28 (C-12), 33.12 (C-13), 21.94 (C-14) and 15.28 (C-15).

9-deoxymuzigadia (20 mg): obtained in the form of mixture with polygodial as jelly material ; soluble in $CHCl_3$; Rf 0.65 (mobile phase hexane: EtOAc, (4:1); IR υ_{cm-1}: 2924 (C-H stretching), 1726 and 1675 cm^{-1} (due to α, β unsaturated carbonyl group), 1675 and 1461 (exocyclic double bond stretching nd bending); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, δ_H 1.62, 1.91(2H, *m,* H-1), 1.71, 1.15 (2H, *m,* H-2), 2.10 (2H, *m*, H-3), 2.1 (1H, *m,* H-5), 2.45 (2H, *m,* H-6), 7.14 (1H, *m,* H-7), 3.01 (1H, *m,* H-9), 9.52 (1H,

ddd, H-11), 9.46 (1H, *s*, H-12), 4.92 (1H, *s,* H_a-13), 4.74 (1H, *s,* H_b-13), 1.01 (3H, *d, J* = 6.8, H-14), 0.74 (3H, *s,* H-15); ^{13}C NMR (100 MHz, CDC_3, chemical shift *δ* in ppm): 39.37 (C-1), 31.55 (C-2), 38.49 (C-3), 151.31 (C-4), 45.75 (C-5), 26.99 (C-6), 153.04 (C-7), 137.88 (C-8), 58.29 (C-9), 38.17 (C-10), 201.24 (C-11), 193.33 (C-12), 106.08 (C-13), 18.40 (C-14) and 13.54 (C-15).

Caseamemin (20 mg): brown jelly material; soluble in $CHCl_3$; Rf 0.5 (mobile phase hexane: EtOAc, (5:1); UV (EtOH) λmax nm: 298; IR υ_{cm-1}: 2916 and 1478 cm^{-1} are due to the C-H stretching and bending, 1376 cm^{-1} geminal dimethyl stretching, 1205 cm^{-1} C-O stretching; ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant J in Hz: δ_H 5.12 (1H, *m,* H-3), 2.14 (2H, *m,* H-4), 6.40 (1H, *d*, *J* =2.8Hz, H-5), 6.49 (1H, *d, J* = 2.8 Hz, H-7), 1.62 (3H, *s*, H-9), 2.14 (3H, *s*, H-10), 2.71 (2H, *t*, *J* = 6.4 Hz, H-1'), 1.74 &1.81 (2H, *td*, H-2'), 2.01 (2H, *m*, H-5'), 1.75 (2H, *m*, H-6'), 5.12 (1H, *m*, H-7'), 1.78 (2H, *m*, H-9'), 2.09 (2H, *m*, H-10'), 5.12 (1H, *m*, H-11'), 1.61 (3H, *s,* H-13'), 1.28 (3H, *s,* H-14'), 1.62 (3H, *d, J* = 3.6 Hz, H-15'), 1.70 (3H, *s*, H-16'); ^{13}C NMR (100 MHz, CDC_3, *δ* in ppm: *δ*c 147.89 (C-2), 124.21 (C-3), 22.20 (C-4), 134.95 (C-4a), 112.63 (C-5), 121.20 (C-6), 115.71 (C-7), 127.30 (C-8), 145.92 (C-8a), 16.0 (C-9), 15.87 (C-10), 22.51 (C-1'), 31.42 (C-2'), 75.31 (C-3'), 39.71 (C-4'), 26.78 (C-5'), 39.71 (C-6'), 124.33 (C-7'), 135.10 (C-8'), 39.71 (C-9'), 26.62 (C-10'), 124.43 (C-11'), 131.31 (C-12'), 25.69 (C-13'), 24.0 (C-14'), 16.0 (C-15') and 16.68, (C-16').

1-heptacosanol (10 mg): oil like jelly material; soluble in $CHCl_3$; Rf 0.68 (mobile phase hexane: EtOAc, (4:1); UV (EtOH) λmax nm: no absorption; IR υ_{cm-1}: 3395 cm^{-1} (O-H), 2924 and 1470 for C-H stretching and bending and 708 for C-C stretching; ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant J in Hz: δ_H 3.66 (2H,*t, J*=6.6 Hz, H-1), 1.59 (2H, *m*, H-2), 1.27 (48H, *br s,*H-3 to H-26), 0.90 (3H, *t*, *J*=6.7 Hz, H-27); ^{13}C NMR (100 MHz, CDC_3): δ_C 63.12 (C-1), 32.83 (C-2), 31.94 (C-3), 29.71-29.37 (C4-C24), 25.76 (C-25), 22.70 (C-26) and 14.11 (C-27).

Hentriacontane (60 mg): white amorphous solid; soluble in $CHCl_3$; Rf 0.75 (mobile phase hexane:$CHCl_3$, (3:2); MS: m/z 436, molecular formula $C_{31}H_{64}$; UV (EtOH) λmax nm: no absorption; mp 58-59^{o}C; IR υ_{cm-1}: 2914 and 1470 due to C-H stretching and bending, 718 (C-C stretching); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm: *δ* 0.90

(6H, *t, J* = Hz, H-1&31) and 1.27 (H-2 up to H-30); ^{13}C NMR (100 MHz, CDC_3): δ_C 14.14 (C-1/31), 22.72 (C2/30), 29.41-29.74 (C3-15/17-29), 31.95 (C-16).

Compound 120 (20 mg): white crystal; soluble in acetone or methanol; Rf 0.40 (mobile phase hexane:EtOAc (1:4)); UV (EtOH) λmax nm: 220; mp 140-142^{o}C; (+)-ESI MS exhibited an ion peak $[M+Na]^+$ at *m/z* = 287 ($C_{15}H_{20}NaO_4$) and [M+H]+ at *m/z* = 265 ($C_{15}H_{21}O_4$) confirming the molecular formula $C_{15}H_{20}O_4$; IR $\upsilon_{cm\text{-}1}$: 3393 due to O-H stretching, 1709 (α, β-unsaturated carbonyl group), 2968 (C-H stretching), 1171 (C-O) and 1636 (C=C); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant in Hz: δ_H 4.73 (1H, *s*) and δ 4.83 (1H, *s*) exocyclic protons, 1.02 (3H, *d, J* = 6.8 Hz) and δ 0.64 (3H, *s*) for two terminal methyl protons, 2.50 (1H, *m,* assigned for methine carbon), 2.23 (2H, *dd, J* = 3.6 Hz), 2.04 1.12 (2H, *m, J = 4 Hz*) 1.64 & 1.08 (2H, *dd, J* = 2.8 and 2.4 Hz); ^{13}C NMR (100 MHz, CDC_3): δ_C two methyl carbons (15.37, 18.99), four methylene carbons (27.12, 31.43, 32.50 and 105.82), methine carbons (38.44, 40.50), four quaternary carbons (167.53, 152.81, 131.50, 75.99).

4.7.3. Extraction and isolation of compounds from *A. aspera* aerial part

Achyranthes aspera aerial part was collected from ALNAP garden, Addis Ababa University. Addis Ababa lies at an elevation of 2,200 metres (7,200 ft) and located at 9°1′48″N 38°44′24″E. The specimen of the plant was deposited at the National Herbarium Department of Biology; Addis Ababa University Herbarium with voucher number of 97-31A and it was identified by a plant taxonomist.

Powder of aerial parts of *A. aspera* (100 g) was soaked in EtOH (300 mL) and left on the shaker for 24 h. The extract was filtered and concentrated to afford EtOH free black jelly material (4 g). The crude extract (3 g) was adsorbed on silica gel (3 g) and applied on silica gel (3 g) column chromatography. 51 fractions were collected and pooled to 14 subfractions on the bases of their TLC profile.

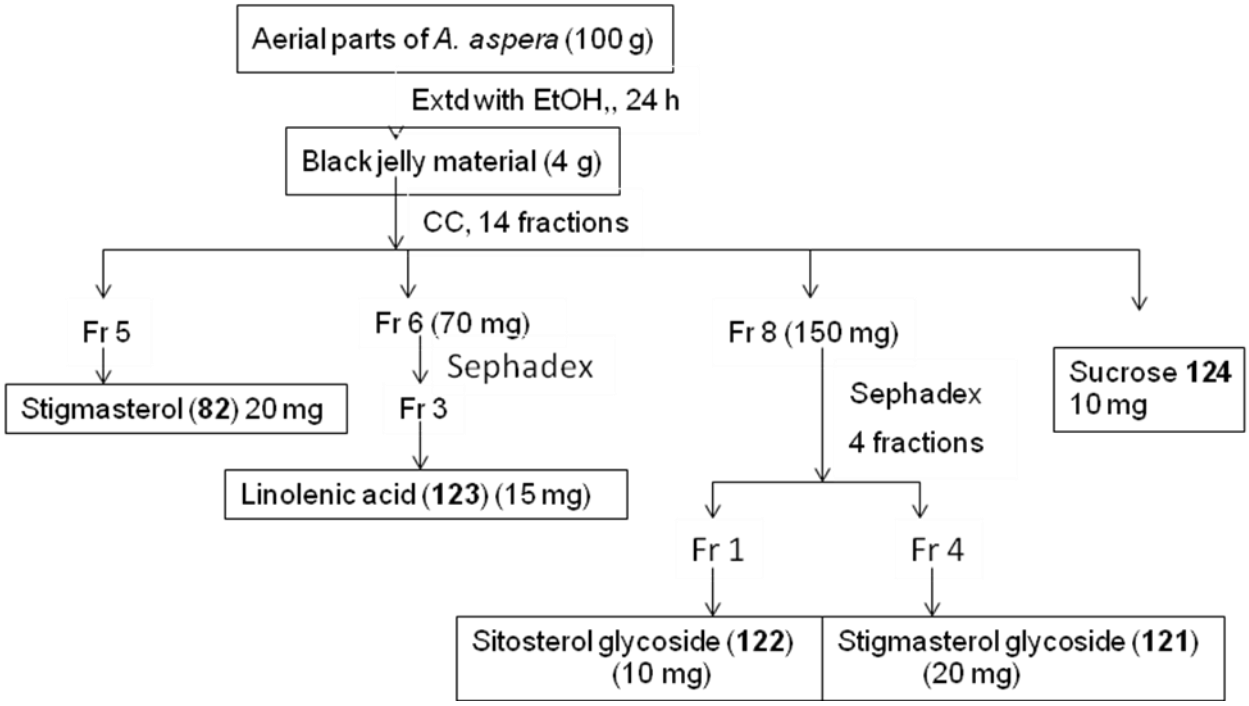
Fraction 2 (50 mg) was dissolved in hexane:EtOAc (4:1) solvent system and left open under the bench. A white oily material (10 mg) was obtained stuck with the vial. It was proposed to be a straight chain alkane.

Fractions 5 (60 mg) was allowed to crystallize using solvent systems of hexane:$CHCl_3$ (2:3) and a white solid material was precipitated out at the bottom of the vial. This white solid was identified to be stigmasterol (20 mg).

Fraction 6 (75 mg) was applied on Sephadex LH20 using $CHCl_3$:MeOH (1:1) solvent system. Three subfractions were collected. Subfraction 3 leads to the isolation of unsaturated fatty acid (linolenic acid) as a brownish powder (15 mg).

Fraction 8 (150 mg) was applied on Sephadex LH20 and elution was made using $CHCl_3$:MeOH (1:1) solvent system. Four subfractions were collected. A white solid material was obtained from subfraction 4. It was identified to be **stigmasterol glycoside** (20 mg). From subfraction 1 white powder was obtained and identified to be ***β*-sitosterol-D-glycoside** (10 mg).

Fraction 14 (200 mg) was dissolved in methanol and white crystal was precipitated out which was identified to be sucrose (10 mg).



Stigmasterol (20 mg, 0.6%): white solid material; soluble in $CHCl_3$; Rf 0.82 (mobile phase hexane:$CHCl_3$:EtOAc (2:2:1)); UV (EtOH) λmax nm: no absorption; mp 140-145^oC; IR υ_{cm-1}: 3430 (O-H), 2938 and 1463 (C-H stretching and bending), 1050 (C-O); ^{1}H-NMR

(400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant in Hz: δ_H 3.51 (1H, *m,* H-3), 5.36 (1H, *t*, *J* = 2.4 Hz, H-6), 0.94 (3H, *d*, *J* = 6.4 Hz, H-19), 5.03 (1H, *m*, H-20), 5.16 (1H, *m,* H-21), 0.86 (3H, *t*, H-24), 0.84 (3H, *d, J* = 7.6 Hz, H-26), 0.82 (3H, *d, J* = 6.8 Hz, H-27), 0.69 (3H, *d*, *J* = 7.6 Hz, H-28), 1.02 (3H, *s*, H-29); ^{13}C NMR (100 MHz, CDC_3), chemical shift value δ in ppm: δ_C 37.27 (C-1), 31.91(C-2), 71.83 (C-3), 42.31 (C-4), 140.75 (C-5), 121.74 (C-6), 31.67 (C-7), 31.90 (C-8), 50.16 (C-9), 36.53 (C-10), 21.10 (C-11), 39.79 (C-12), 42.20 (C-13), 56.88 (C-14), 24.33 (C-15), 29.15 (C-16), 56.02 (C-17), 40.54 (C-18), 21.25 (C-19), 138.34 (C-20), 129.26 (C-21), 45.84 (C-22), 25.44 (C-23), 12.01 (C-24), 28.28 (C-25), 19.81 (C-26), 19.43 (C-27), 19.05 (C-28), 12.29 (C-29).

Stigmasterol glycoside (20 mg): white solid material; soluble in MeOH, DMSO; Rf 0.61 (mobile phase formic acid:EtOAc:MeOH (1:2:3)); UV (EtOH) λmax nm: no absorption; mp 260-262^{o}C; IR υ_{cm-1}: 3428 (O-H), 1625 (C=C), 2916 (C-H) and 1023 (C-O); ^{1}H-NMR (400 MHz, DMSO), chemical shift δ in ppm, coupling constant in Hz: δ_H 3.55 (1H, *m,* H-3), 5.33 (1H, *d, J* = 2 Hz, H-6*)*, 0.50 (3H, *s,* H-18), 0.96 (3H, *s*, H-19), 0.99 (3H, *d, J* = 6 Hz, C-21), 5.12 (1H, *dd,* H-22), 5.02 (1H, *dd,* H-23) 0.82 (3H, *d*, J = 7.2 Hz, C-26), 0.78 (3H, *d*, *J* = 4 Hz, H-27), 0.89 (3H, *d, J* = 6 Hz, H-29), 4.21 (1H, *d*, J = 7.6 Hz, H-1'), 3.36 (2H, *m*, H-6'); ^{13}C NMR (100 MHz, DMSO): δ_C 37.31 (C-1), 31.90 (C-2), 77.41 (C-3), 42.23 (C-4), 140.94 (C-5), 121.70 (C-6), 29.74 (C-7), 31.86 (C-8), 50.11 (C-9), 36.70 (C-10), 21.43 (C-11), 38.79 (C-12), 42.34 (C-13), 56.66 (C-14), 24.34 (C-15), 29.19 (C-16), 55.91 (C-17), 12.16 (C-18), 19.33 (C-19), 45.62 (C-20), 20.21 (C-21), 139.54 (C-22), 129.30 (C-23), 51.08 (C-24), 31.90 (C-25), 19.59 (C-26), 19.42 (C-27), 25.36 (C-28), 12.27 (C-29), 101.32 (C-1'), 73.95 (C-2'), 76.77 (C-3'), 70.57 (C-4'), 77.17 (C-5'), 61.57 (C-6').

***β*-sitosterol-D-glycoside (10 mg)**: white solid material; soluble in MeOH, DMSO; Rf 0.55 (mobile phase formic acid:MeOH (1:4)); UV (EtOH) λmax nm: no absorption; ^{1}H-NMR (400 MHz, DMSO), chemical shift δ in ppm, coupling constant in Hz: δ_H 4.44 (1H, *m,* H-3), 5.33 (1H, *t,* H-6), 0.65 (3H, *s,* H-18), 0.95 (3H, *s,* H-19), 0.90 (3H, *d, J* = 6 Hz, H-21), 0.78 (3H, *d, J* = 2.8 Hz, H-26), 0.74 (3H, s, H-27), 1.23 (2H, *m*, H-28), 0.80 (3H, *t*, *J* = 6.4 Hz, H-29), 4.21 (1H, *d, J* = 8 Hz, H-1'), 3.62 (2H, *m,* H-6'); ^{13}C NMR (100 MHz, DMSO): δ_C 37.32 (C-1), 31.82 (C-2), 77.39 (C-3), 42.34 (C-4), 140.92 (C-5), 121.65 (C-6), 31.86 (C-7), 31.90 (C-8), 50.09 (C-9), 36.70 (C-10), 20.20 (C-11), 38.79 (C-12), 42.23 (C-13), 56.66 (C-14), 24.35 (C-15), 29.20 (C-16), 55.91 **(**C-17), 12.16 (C-18), 19.59 (C-19), 35.97

(C-20), 19.43 (C-21), 33.83 (C-22), 25.36 (C-23), 45.63 (C-24), 29.75 (C-25), 19.34 (C-26), 19.11 (C-27), 23.10 (C-28), 12.27 (C-29), 101.26 (C-1'), 73.59 (C-2'), 77.18 (C-3'), 70.53 (C-4'), 77.39 (C-5'), 61.58 (C-6').

Linolenic (9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic acid (15 mg): light yellowish powder; Rf 0.24 (mobile phase $CHCl_3$:EtOAc, 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift *δ* in ppm, coupling constant in Hz: δ_H 5.38: multiplet downfield signal appeared at *δ* indicative for protons at a double bond position, 0.99 (3H, *t*, terminal homoallylic protons), 2.35 (2H, *t,* allylic methylene protons), 1.65 (2H, m, methylene protons β to carboxylic acid), 2.81 (protons attached to the *bis*-allylic carbons), 1.27 (methylene protons in the fatty acid chain); ^{13}C NMR (100 MHz, $CDCl_3$, *δ* in ppm: *δ*c 179.20 (carbonyl carbon of the acid functional group), 131.98, 130.27, 128.30, 128.26, 127.76, 127.1(olefinic carbons of the fatty acid), 14.31(terminal methyl carbon), 20.58, 24.69, 25.55, 25.64, 27.22, 29.05, 29.10, 29.17, 29.75, 33.91.

Sucrose (10 mg): white crystal; soluble in H_2O; TLC developed using solvents as a mobile phase, MeOH:EtOAc: AcOH; ^{1}H-NMR (400 MHz, D_2O), chemical shift *δ* in ppm, coupling constant in Hz: δ_H 5.41(1H, *d, J*= 3.6 Hz, anomeric proton), the remaining protons appeared in the region between 3.32 and 4.64; ^{13}C NMR (100 MHz, D_2O : displayed 12 carbons, 103.93 (anomeric carbon), 92.27, 82.35, 77.93, 74.31, 73.21, 73.00, 71.80, 69.94, 62.61, 62.02 and 60.80.

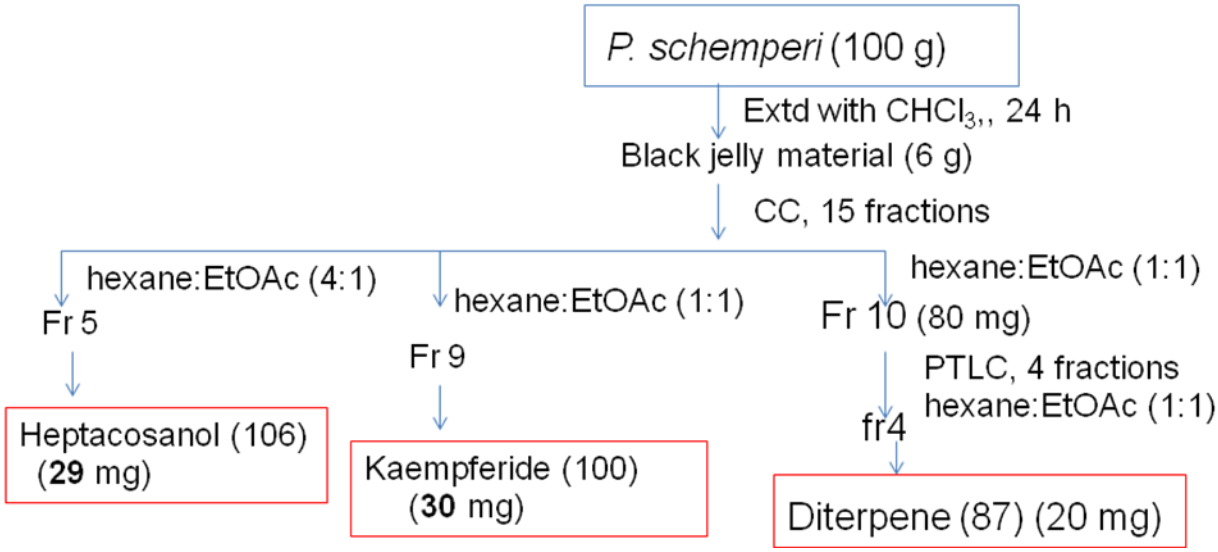
4.7.4. Extraction and isolation of compounds from *P. schimperi* leaves

Premna schimperi leaves were collected from Ambo, west shewa zone of Oromia region. The town has a latitude and longitude of 8°59′N 37°51′E. It is found at elevation of 2101 meters. The specimen was identified by a plant taxonomist and it was deposited at the National Herbarium Department of Biology; Addis Ababa University Herbarium with voucher number of 97-9G.

Based on the preliminary biological activity results of *Premna schimperi*, three compounds were isolated from the $CHCl_3$ extracts of the aerial part of the plant. Powder of *P. schimperi* leaves (100 g) was extracted with $CHCl_3$ (500 mL) to afford 6 g black jelly material. It was adsorbed with silica gel and applied on silica gel (100 g)

column chromatography. Elution was made using solvents of hexane:EtOAc of increasing polarities to furnish 15 fractions. Fraction 5, which was collected using hexane:EtOAc (4:1) solvent system as eluent solvent, allowed to stay overnight and a white powder was formed at the bottom of the vial. The structure of this substance was proposed to be Heptacosanol (29 mg) using different physical and spectroscopic data. Fraction 9 yielded yellow powder in hexane:EtOAc (1:1) solvent system. The powder was subjected to physical and spectroscopic examination and identified to be kaempferide (30 mg). Fraction 10 (80 mg) was applied on PTLC using hexane:EtOAc (1:1) as eluent solvent and four fractions were collected. Fraction 4 was identified to be diterpene compound (20 mg).

Elucidation and characterization of the compounds isolated (labdane diterpene (**99**), kaempferide (**1112**) and 1-heptacosanol (**118**) were carried on the bases of physical and spectroscopic analysis and the data is compared with the literature values.



Diterpene (20 mg): jelly material; soluble in $CDCl_3$; Rf 0.54, mobile phase hexane:EtOAc (3:2); UV (EtOH) λmax nm: no absorption in the UV and Visible region; IR υ_{cm-1}: 3429 cm^{-1} (O-H stretching), 2924 cm^{-1}, (C-H stretching), 1632 cm^{-1} (carbonyl functional group), 1384 cm^{-1} (geminal dimethyl stretching); ^{1}H-NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant in Hz: δ_H: 5.15 (1H, *s*, H-3), 1.96 (1H, *m*, H-8), 1.68 (1H, *dd, J* = Hz, H-11), 2.85, 2.61 (2H, *dd*, *J* = 15.2 Hz, H-11), 3.30 (2H, *s,* H-14), 5.92 (1H, *s, H_a-16*), 6.19 (1H, *s,* H_b-16), 0.86 (3H, *d, J* = 6.8 Hz, H-17), 1.57 (3H, *s,* H-18) 0.96 (3H, *s,* H-19),

0.82 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): 19.63 (C-1), 36.19 (C-2), 120.53 (C-3), 143.32 (C-4), 38.36 (C-5), 27.47 (C-6), 26.53 (C-7), 37.14 (C-8), 42.18 (C-9), 46.61(C-10), 43.38 (C-11), 201.32 (C-12), 143.80 (C-13), 36.17 (C-14), 176.16 (C-15), 126.62 (C-16), 17.62 (C-17), 19.93 (C-18), 17.99 (C-19) and 16.61 (C-20).

Kaempferide (30 mg): yellow powder; soluble in MeOH, DMSO; Rf 0.51, mobile phase hexane:EtOAc (3:2); mp: 219-220^{o}C; UV (EtOH) λmax nm: 268 and 364; IR υ_{cm-1}: 3301, 3455 and 3515 cm^{-1} each assigned for O-H stretching of a hydroxyl group of a flavonoid found in different environment, 1667 (carbonyl group), 1512 (C=C stretching of the aromatic group), 1162 cm^{-1} C-O stretching; ^{1}H-NMR (400 MHz, DMSO-d6), chemical shift *δ* in ppm, coupling constant in Hz: δ_H 6.20 (1H, *s*, H-6), 6.45 (1H, *s*, H-8), 8.12 (1H, *d, J* = 8.8 Hz, H-2'), 7.09 (1H, *d, J* = 9.2 Hz, H-3'), 7.09 (1H, *d, J* = 9.2 Hz, H-5'), 8.14 (1H, *d, J* = 8.8 Hz, H-6'), 3.83 (3H, *s*, methyl of the methoxy group), 9.47 (1H, *s*, 3-OH), 12.45 (1H, *s*, 5-OH), 10.83 (1H, *s*, 7-OH); ^{13}C NMR (100 MHz, DMSO-d6): 146.44 (C-2), 136.52 (C-3), 176.45 (C-4), 161.18 (C-5), 98.70 (C-6), 164.44 (C-7), 93.99 (C-8), 156.69 (C-9), 103.55 (C-10), 123.71 (C-1'), 129.78 (C-2'), 114.48 (C-3'), 160.93 (C-4'), 114.48 (C-5'), 129.78 (C-6'), 55.80 (O-CH_3).

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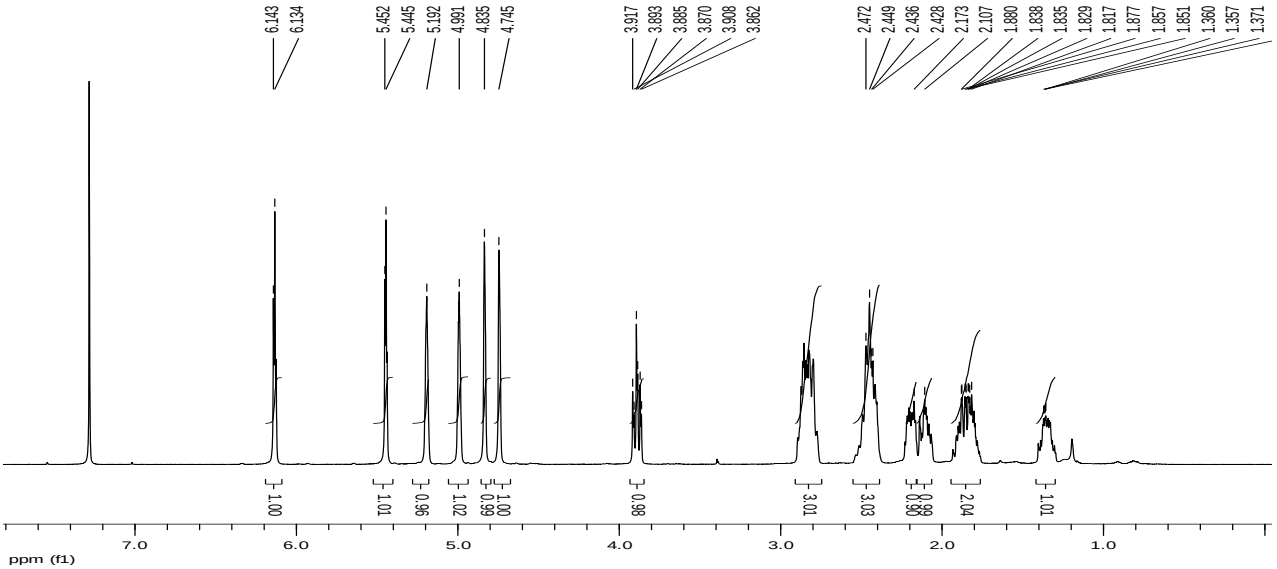
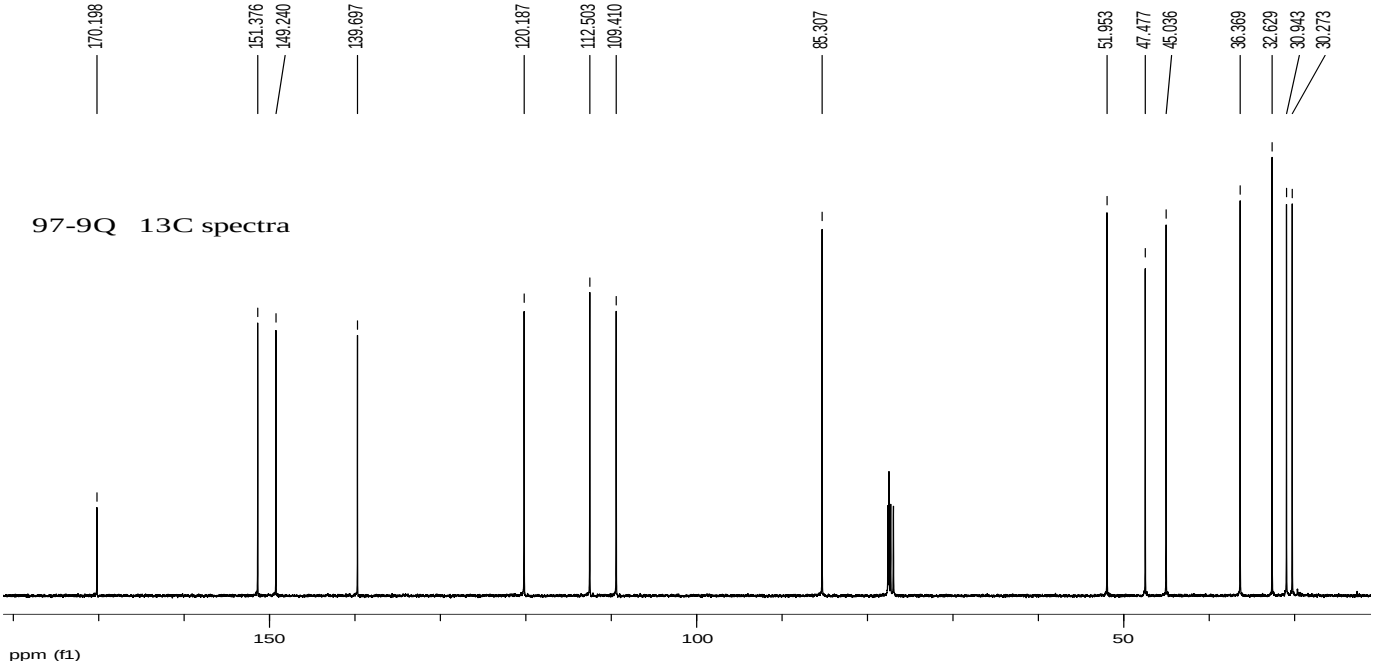
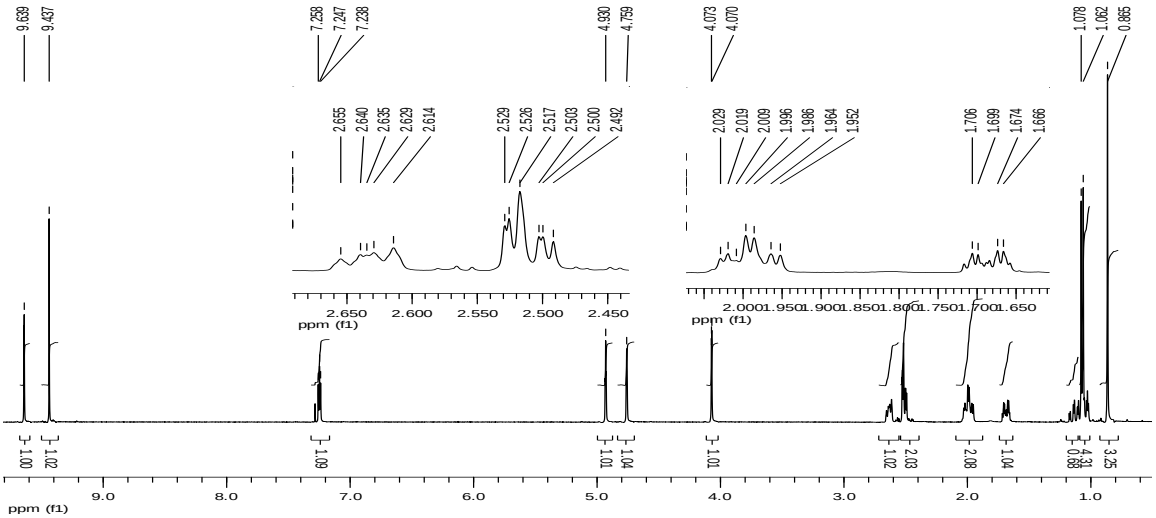
6. Appendices

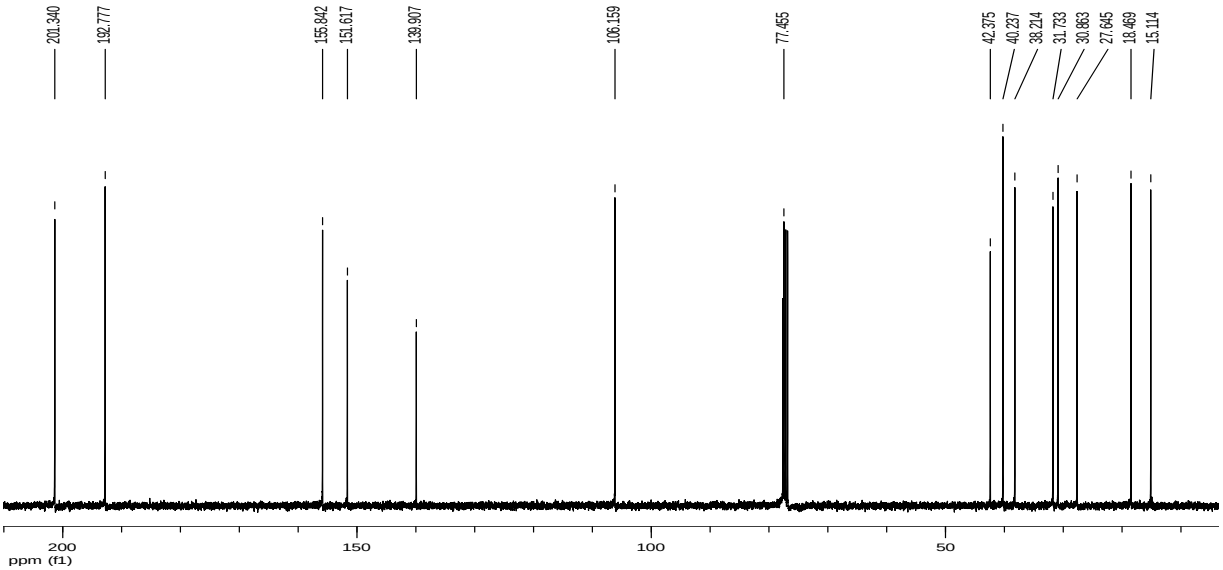
Appendix 1: NMR spectral data of dehydrocostus lactone ($CDCl_3$)

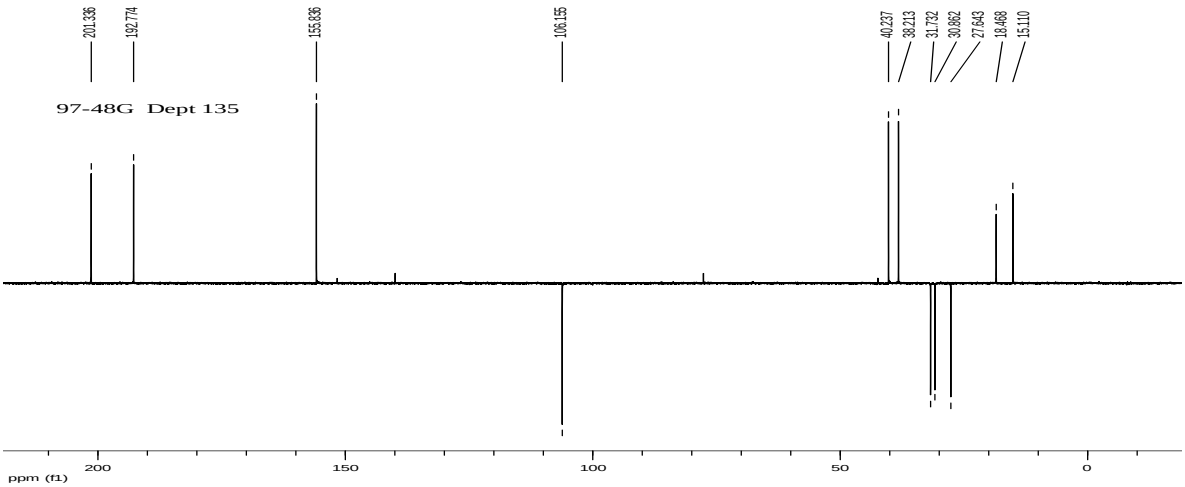




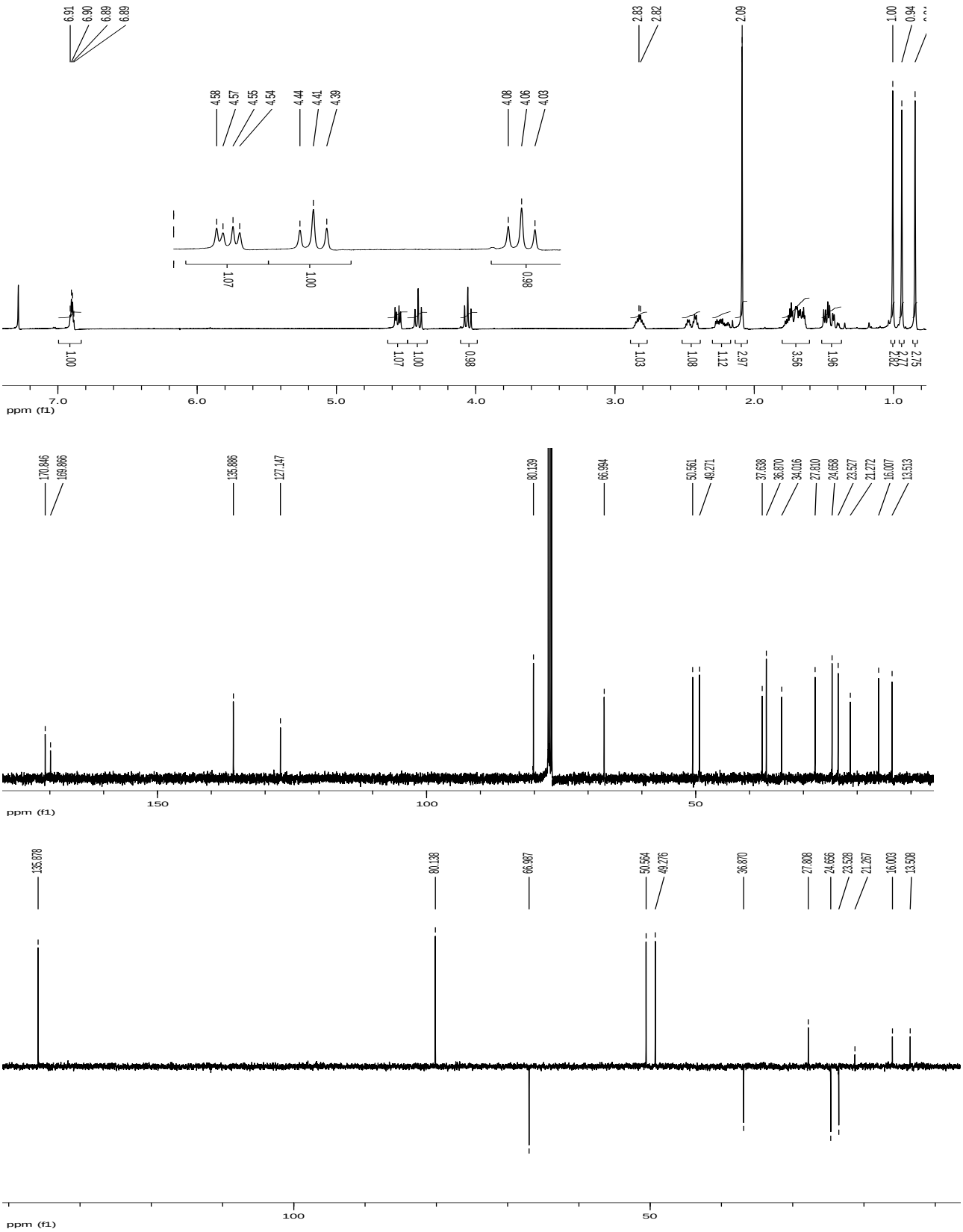
Appendix 2: NMR spectral data of muzigadial ($CDCl_3$)



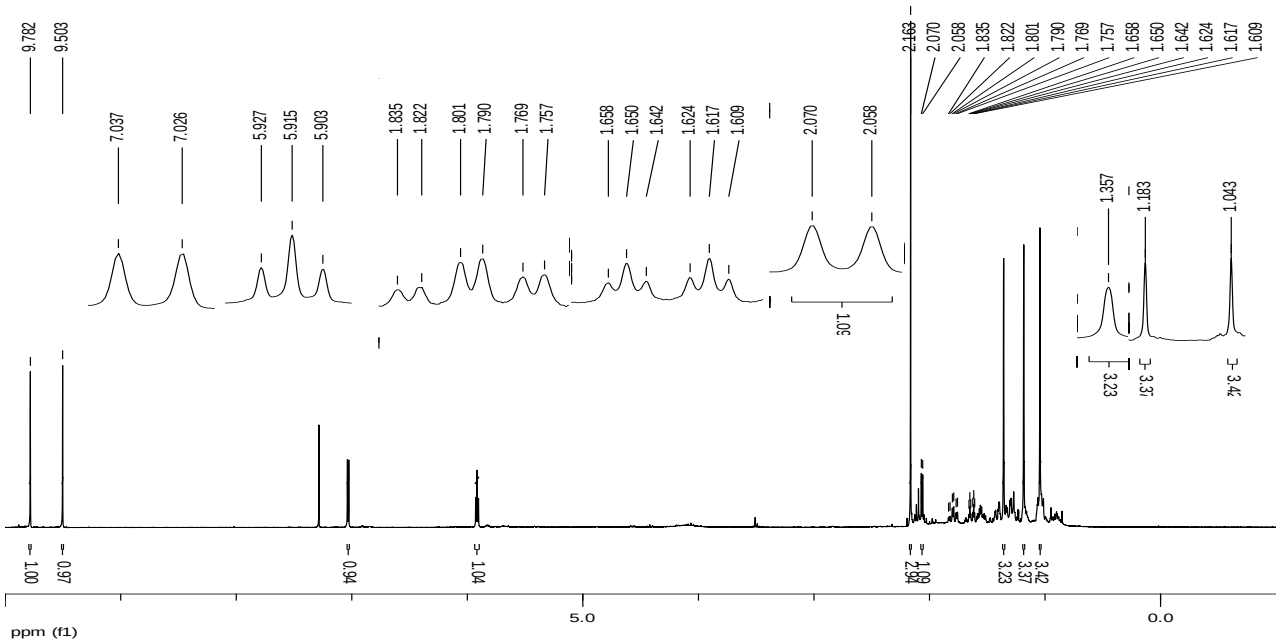


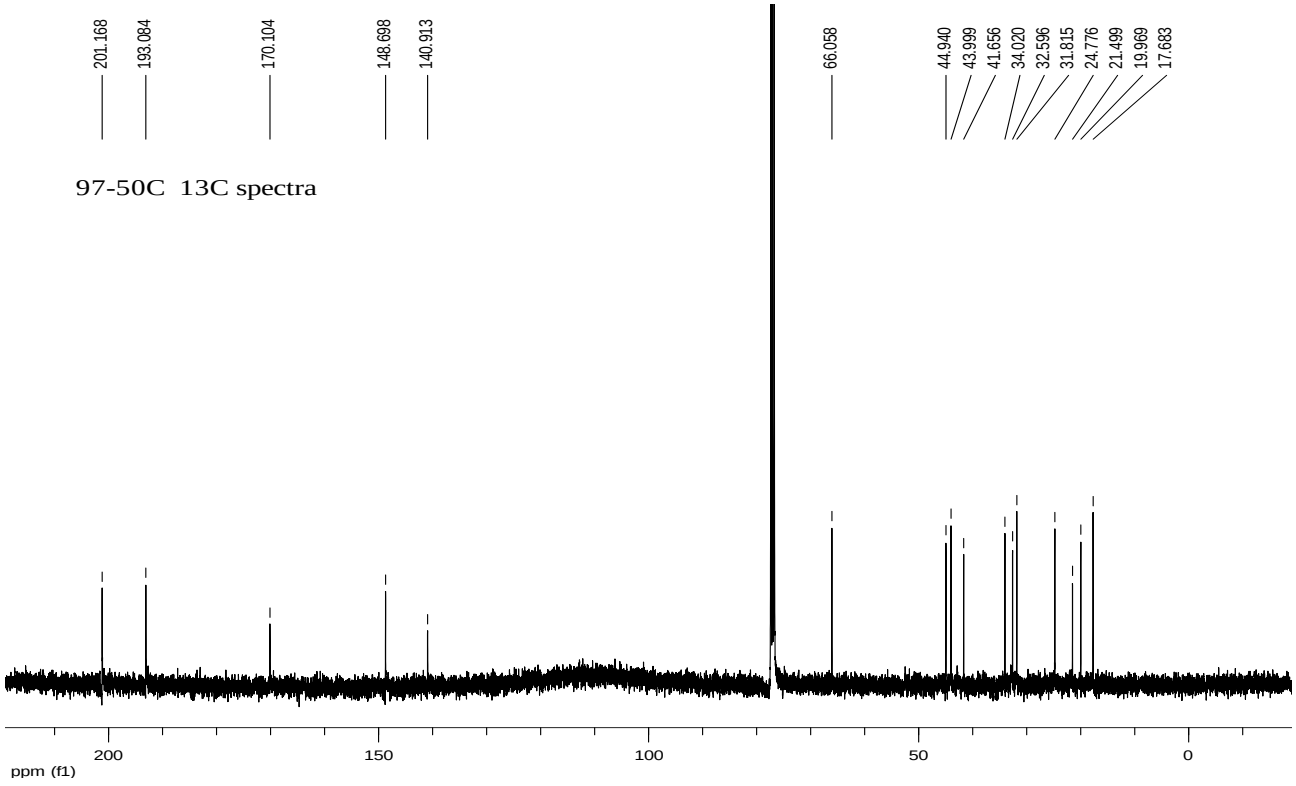


Appendix 3: NMR spectral data of cinnamolide-3β-acetate (CDCl3)

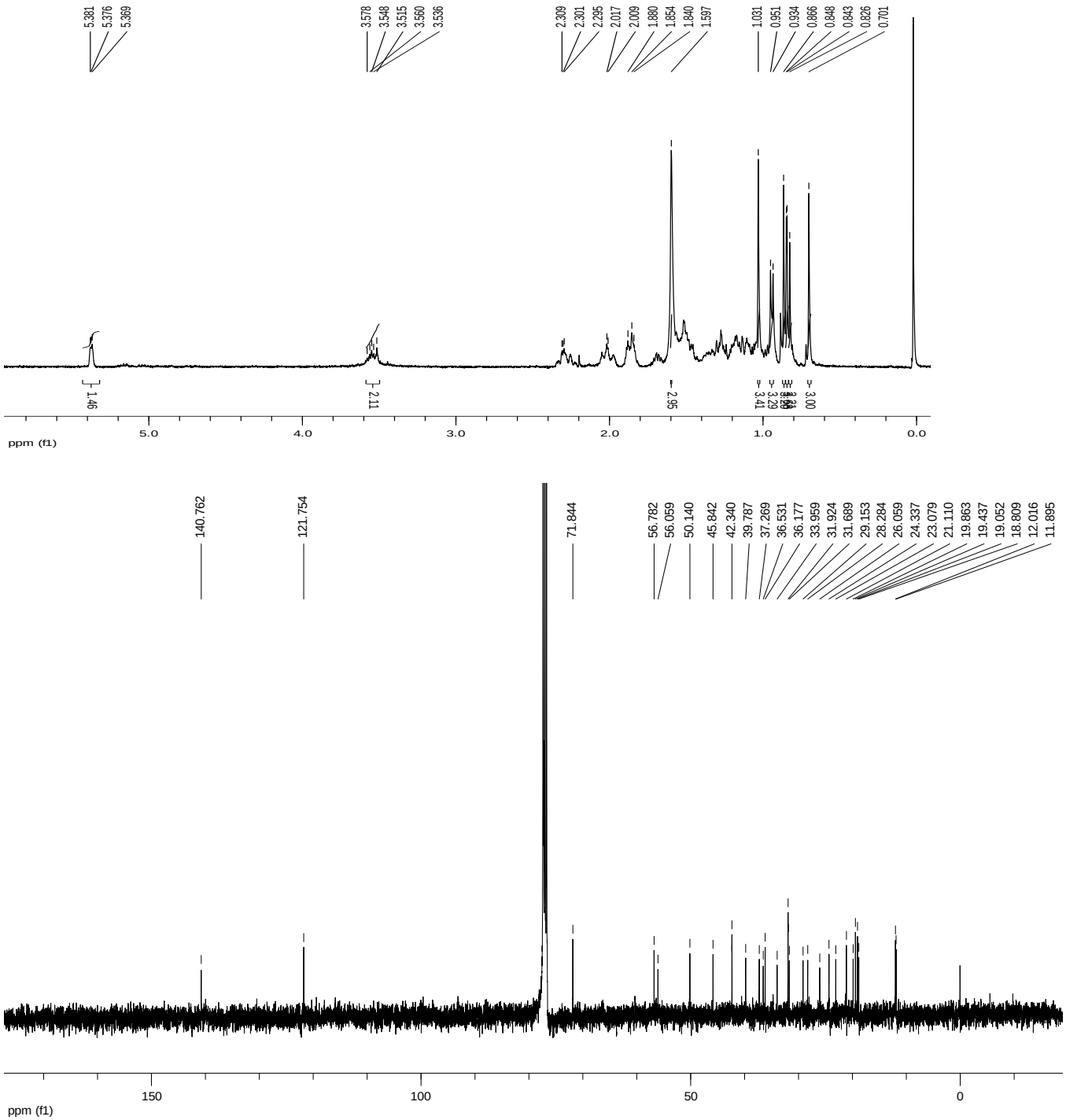


Appendix 4: NMR spectral data of Ugandensidial or cinnamodial ($CDCl_3$)

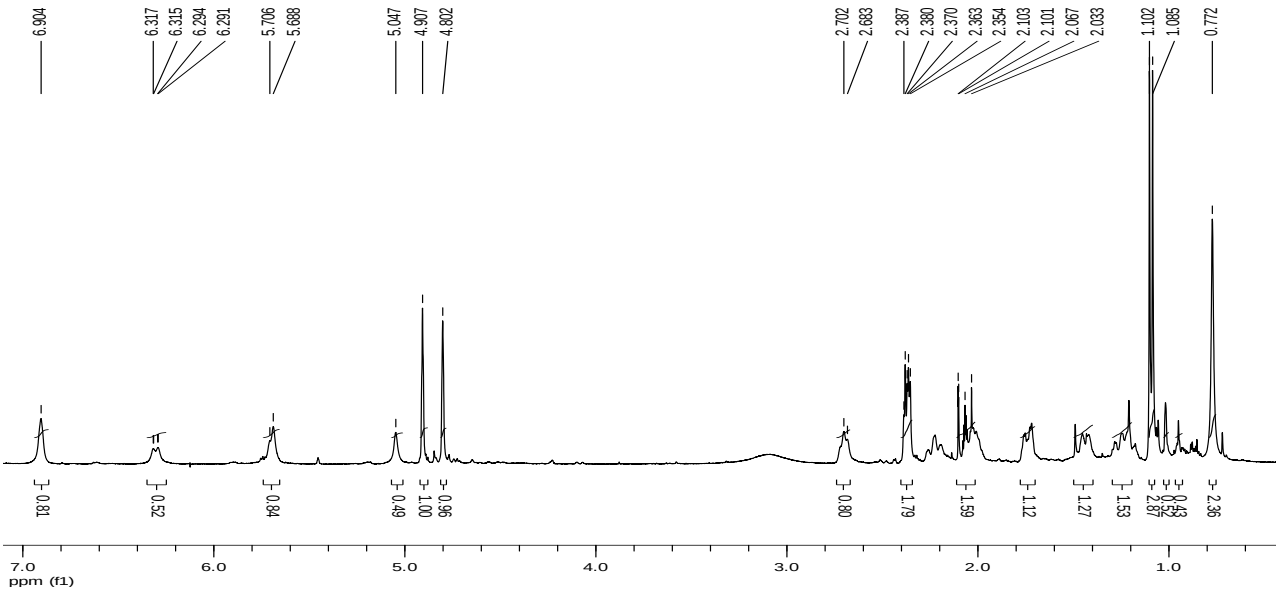


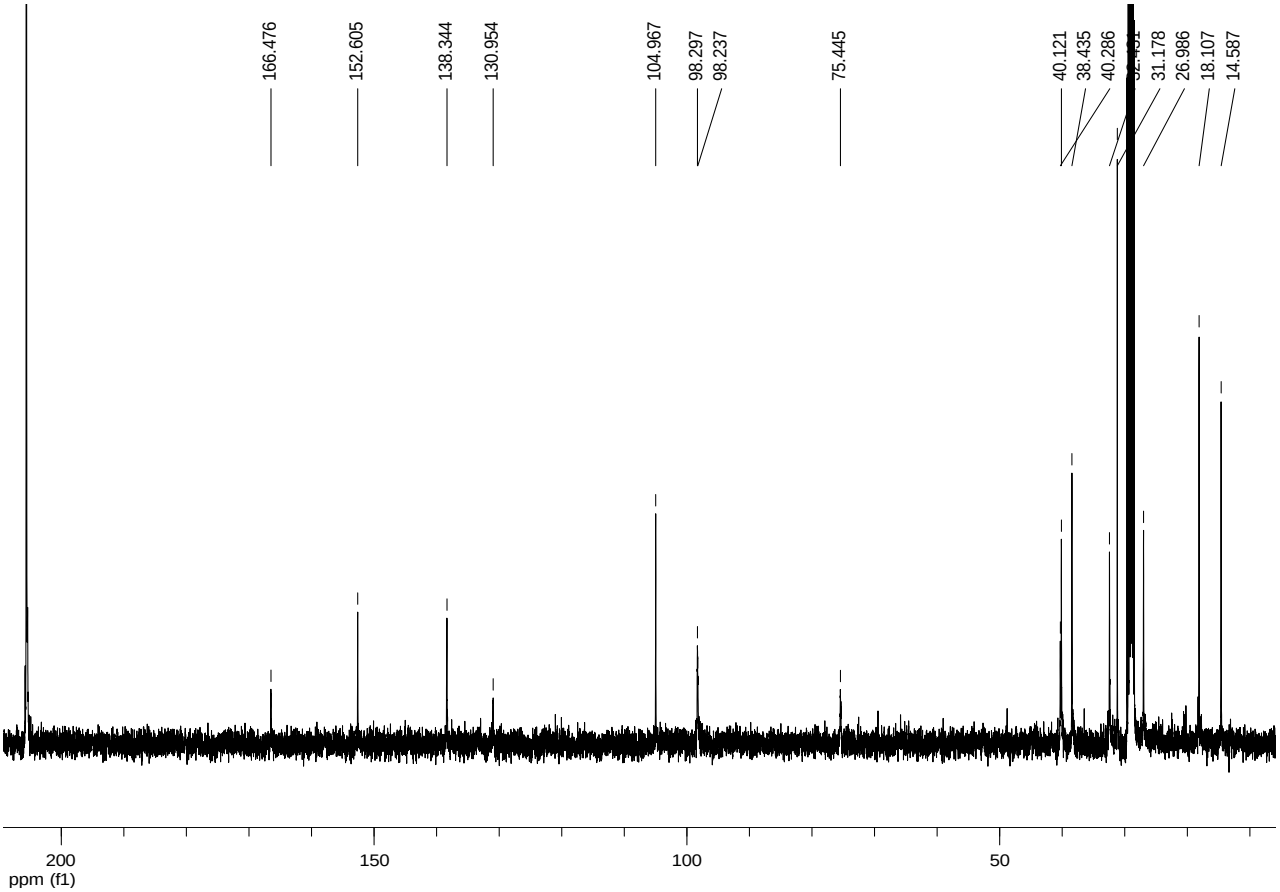


Appendix 5: NMR spectral data of β-sitosterol ($CDCl_3$)

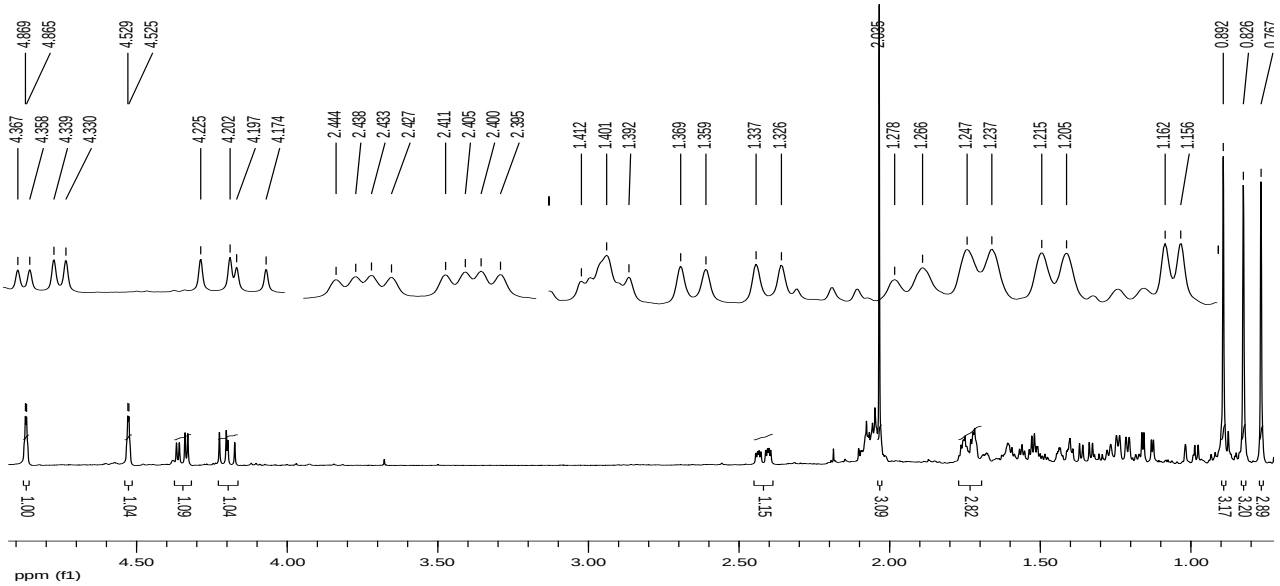


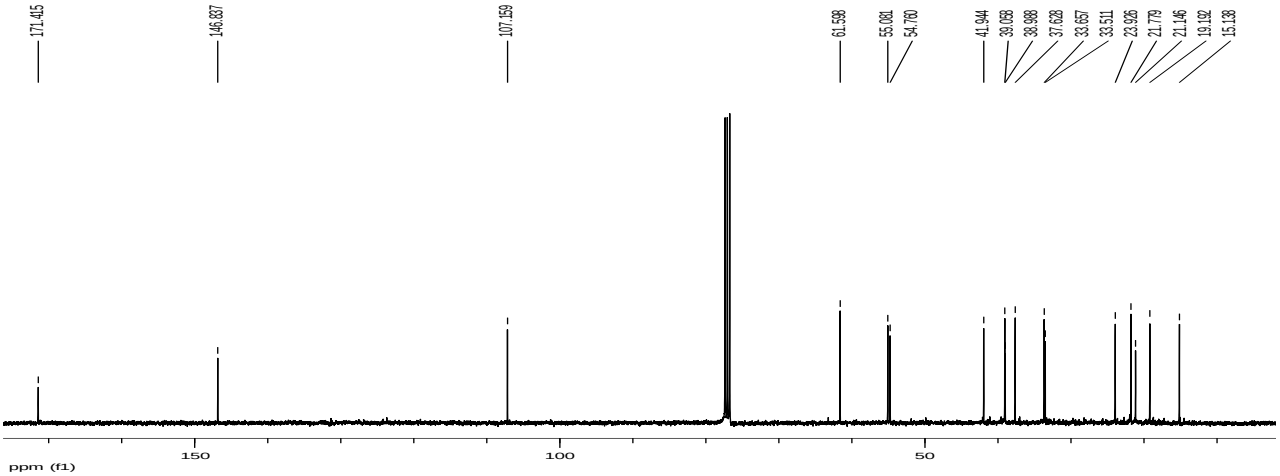
Appendix 6: NMR spectral data of 11α-hydroxy muzigadiolide (Acetone-d6)

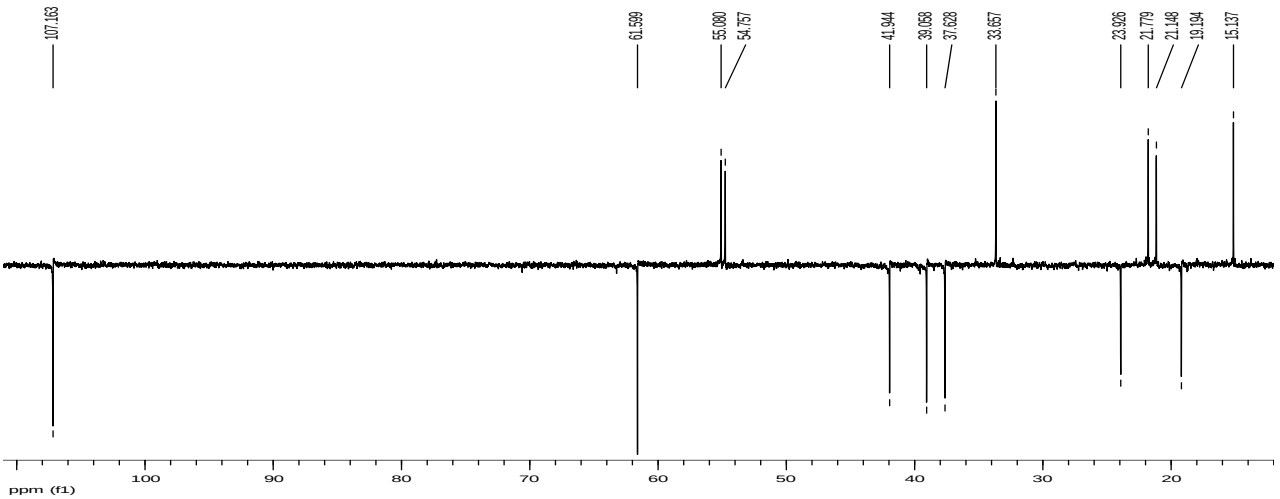




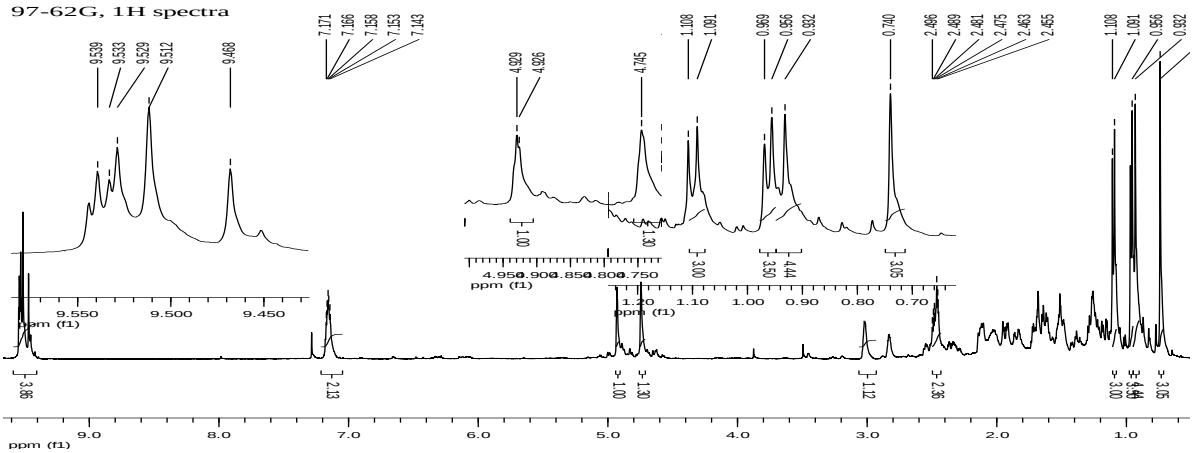
Appendix 7: NMR spectral data of albicanyl acetate ($CDCl_3$)

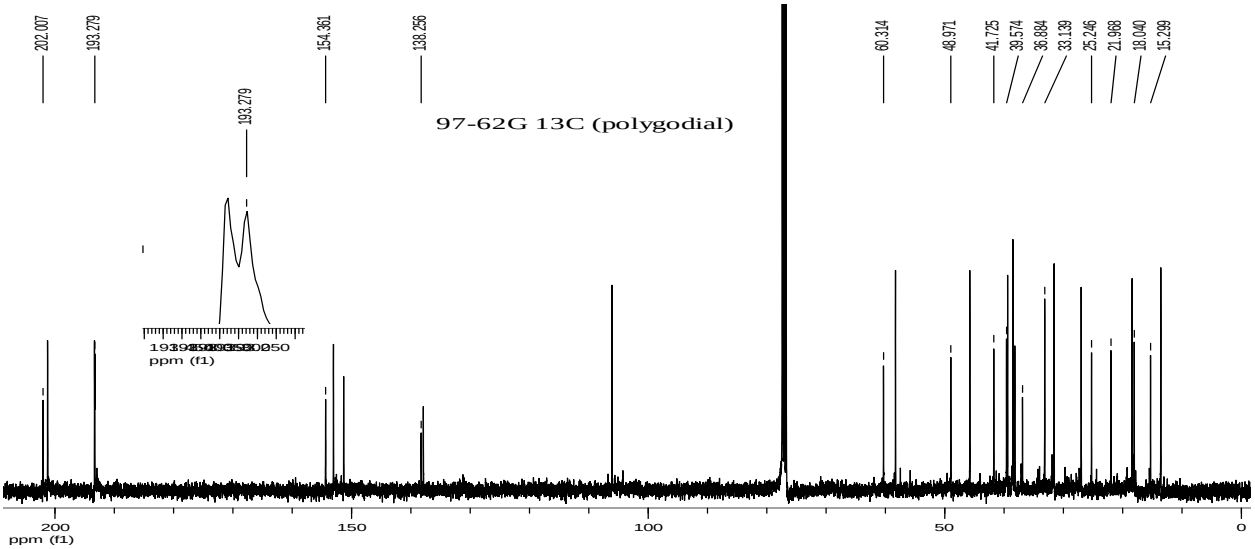


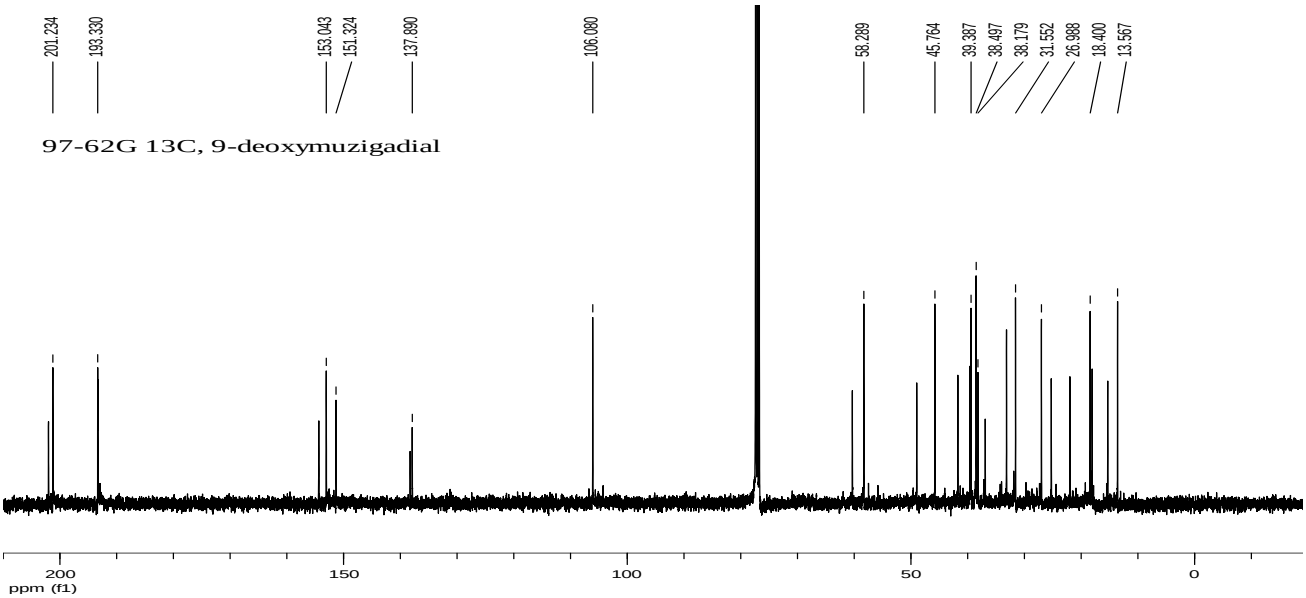




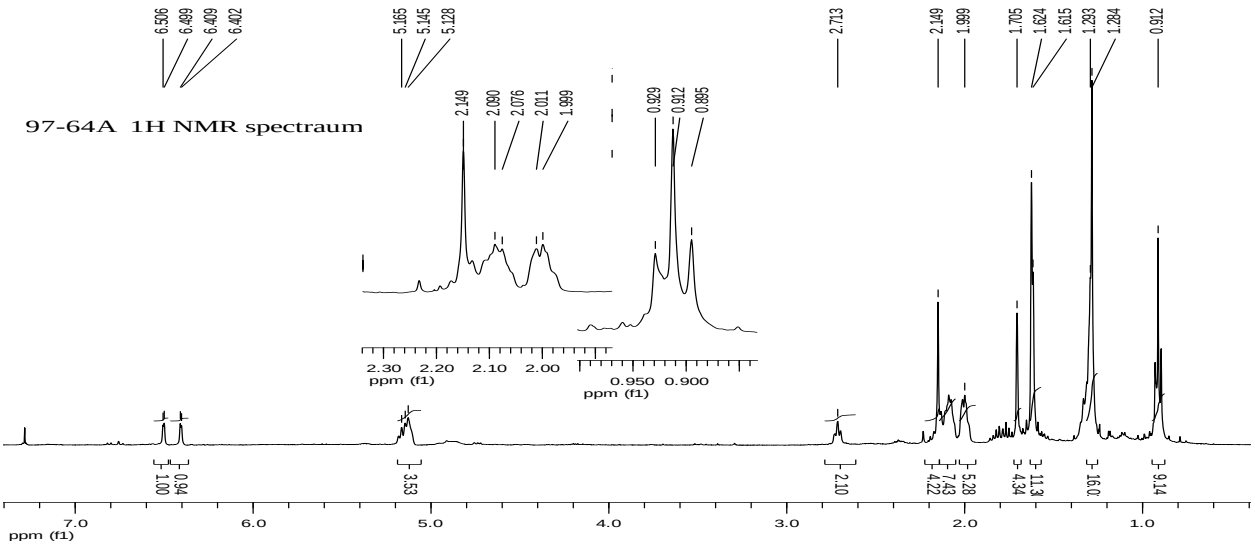
Appendix 8: NMR spectral data of mixtures of polygodial 9-deoxymuzigadial ($CDCl_3$)

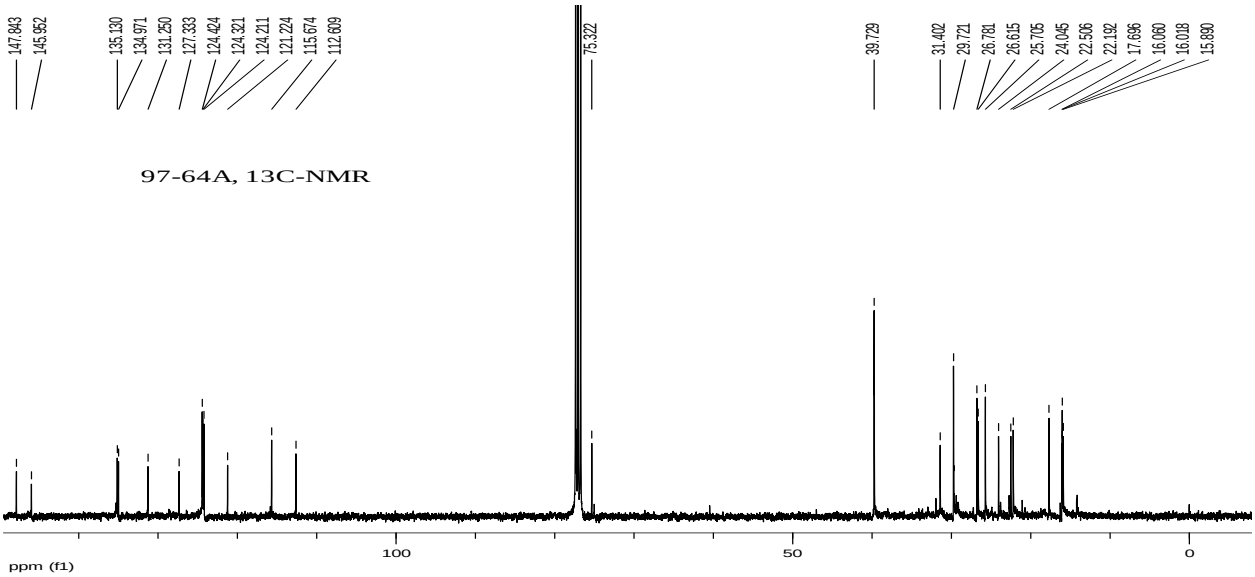


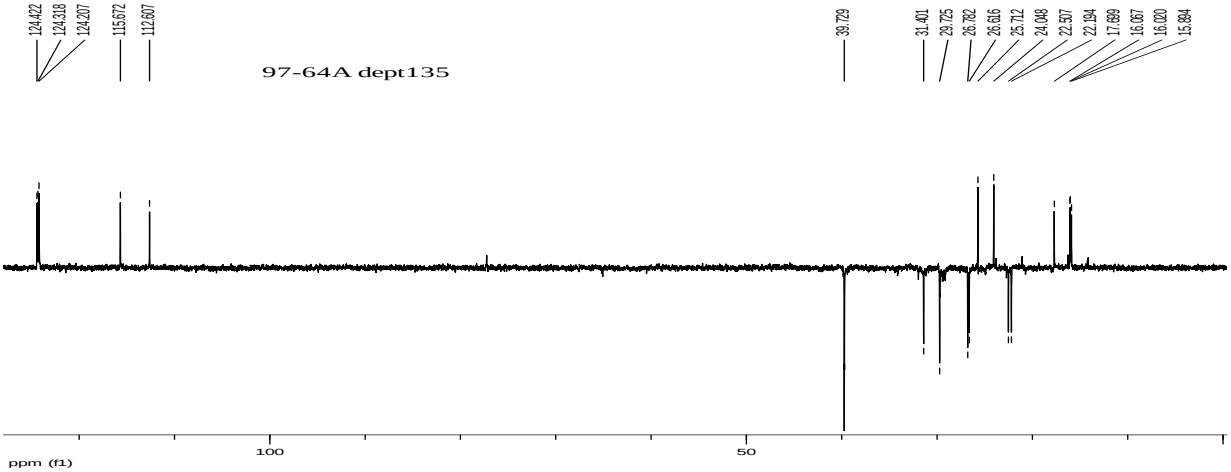




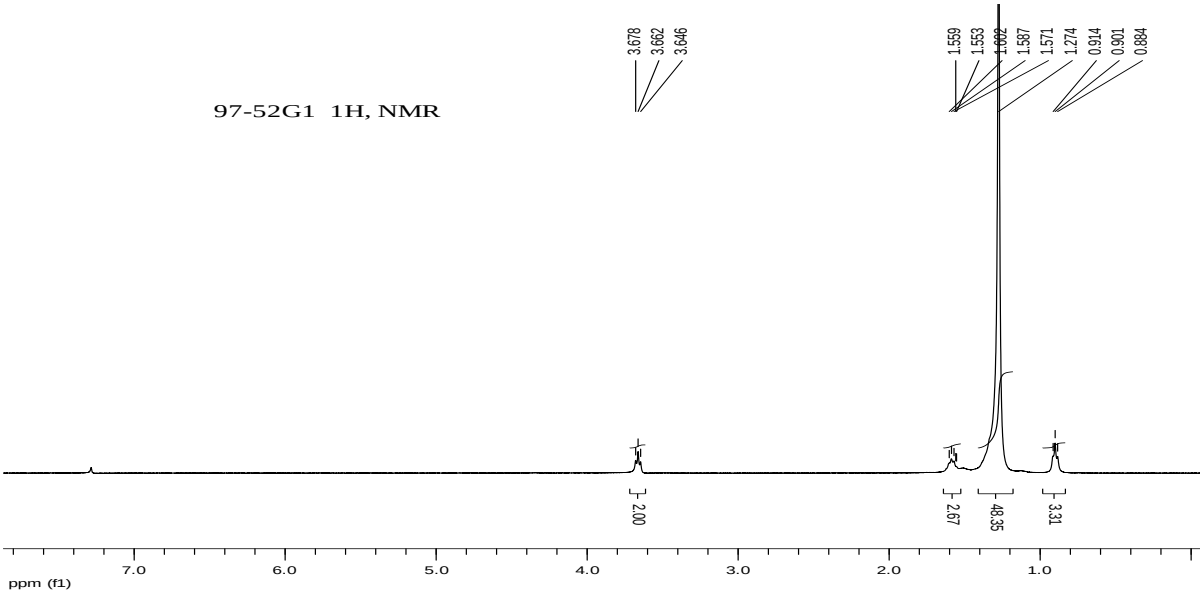
Appendix 9: NMR spectral data of caseamemic ($CDCl_3$)

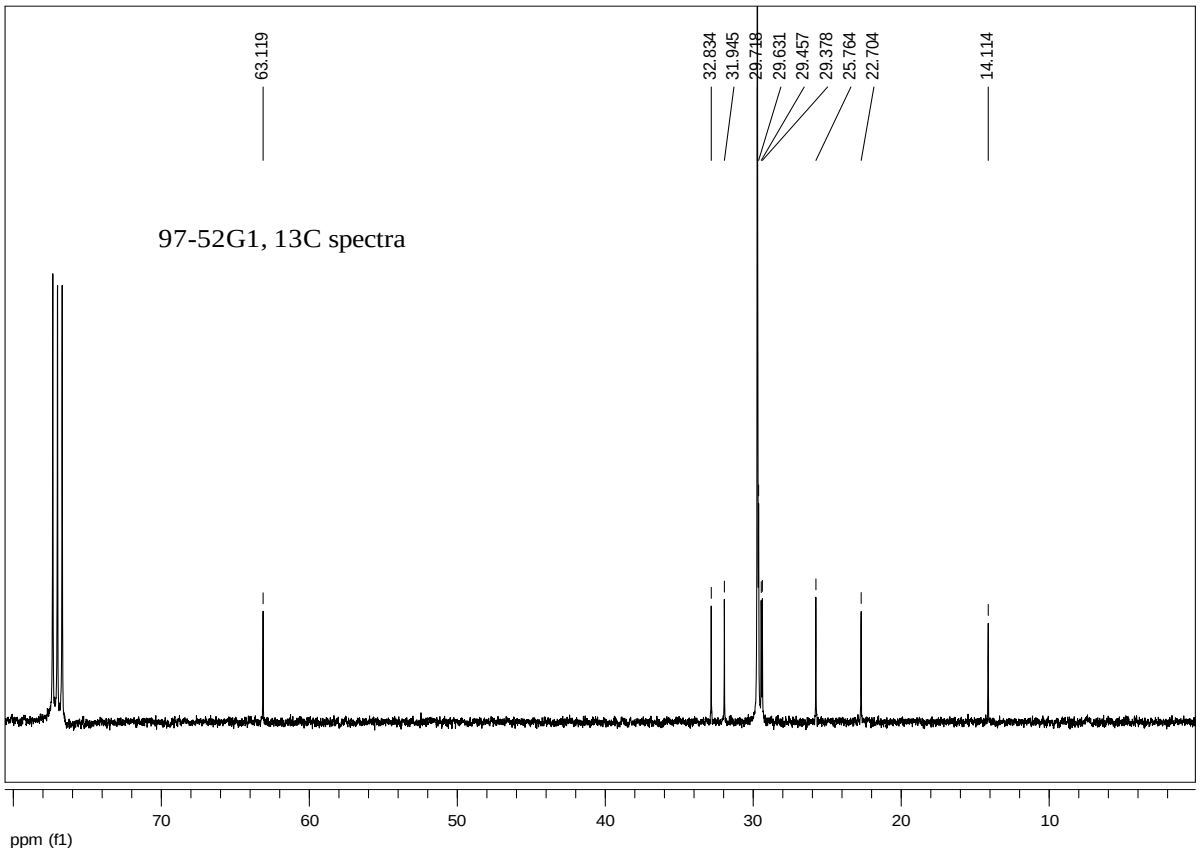




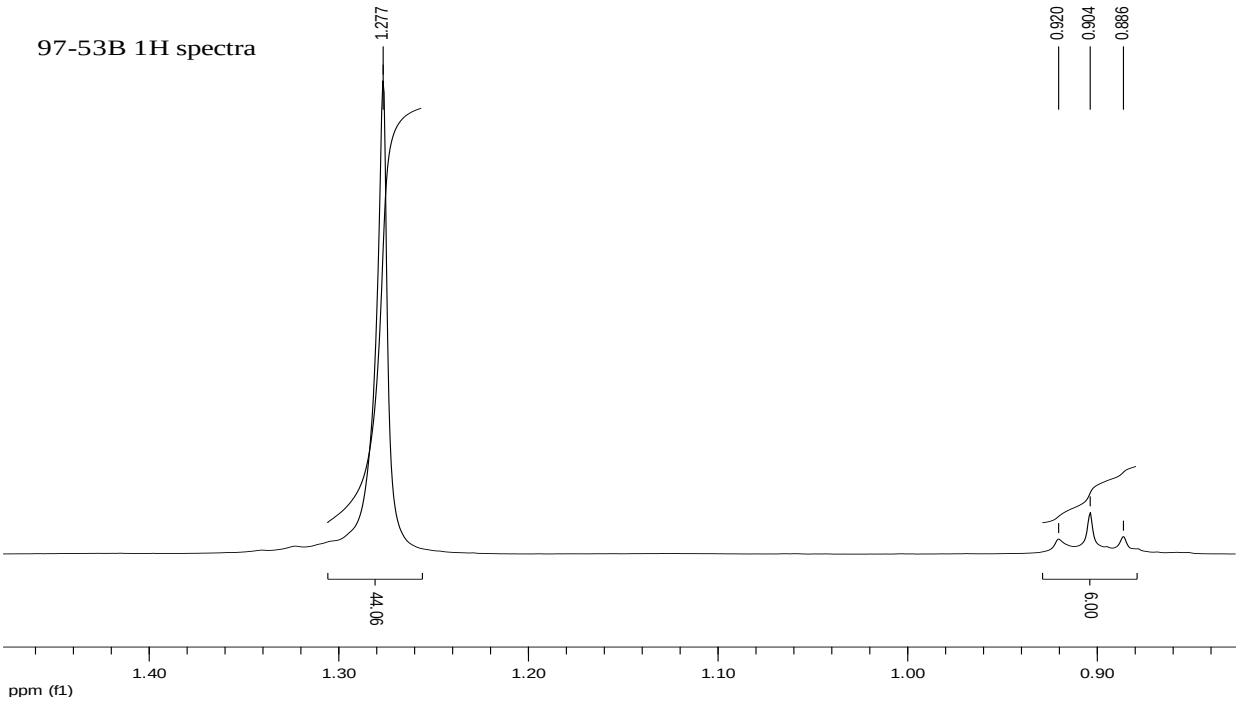


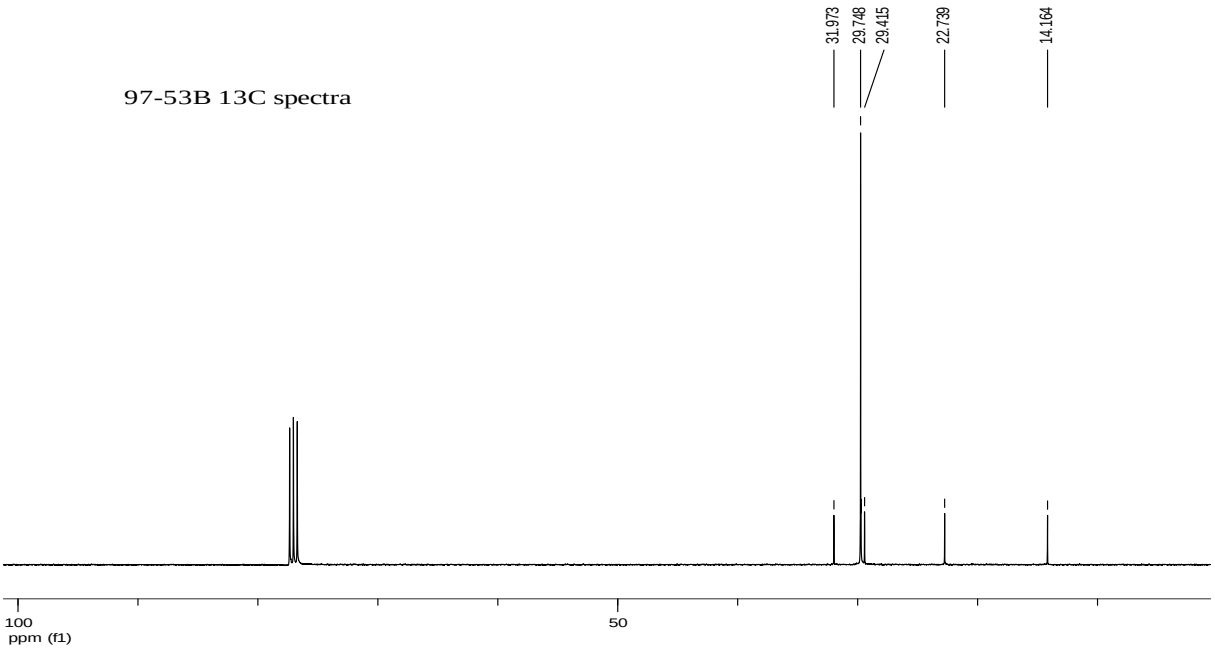
Appendix 10: ^{1}H NMR spectra of 1-heptacosanol ($CDCl_3$)



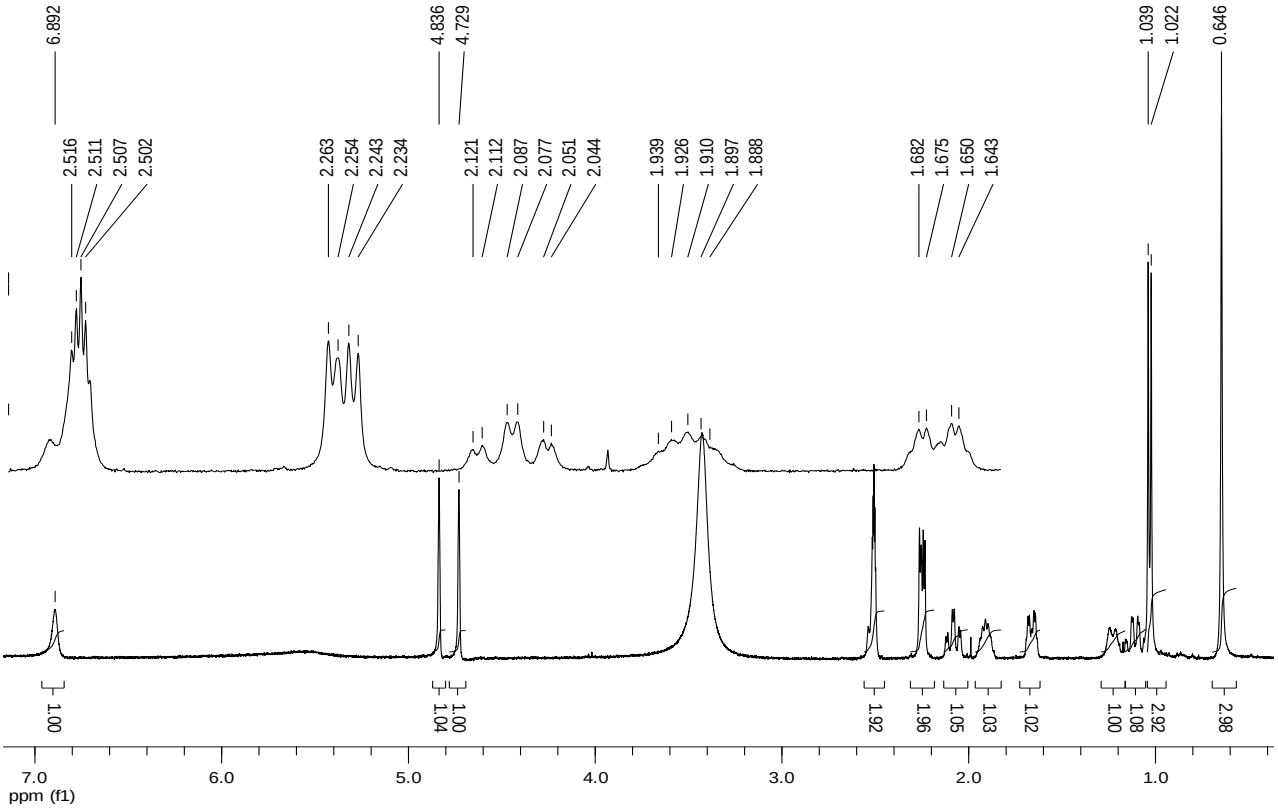


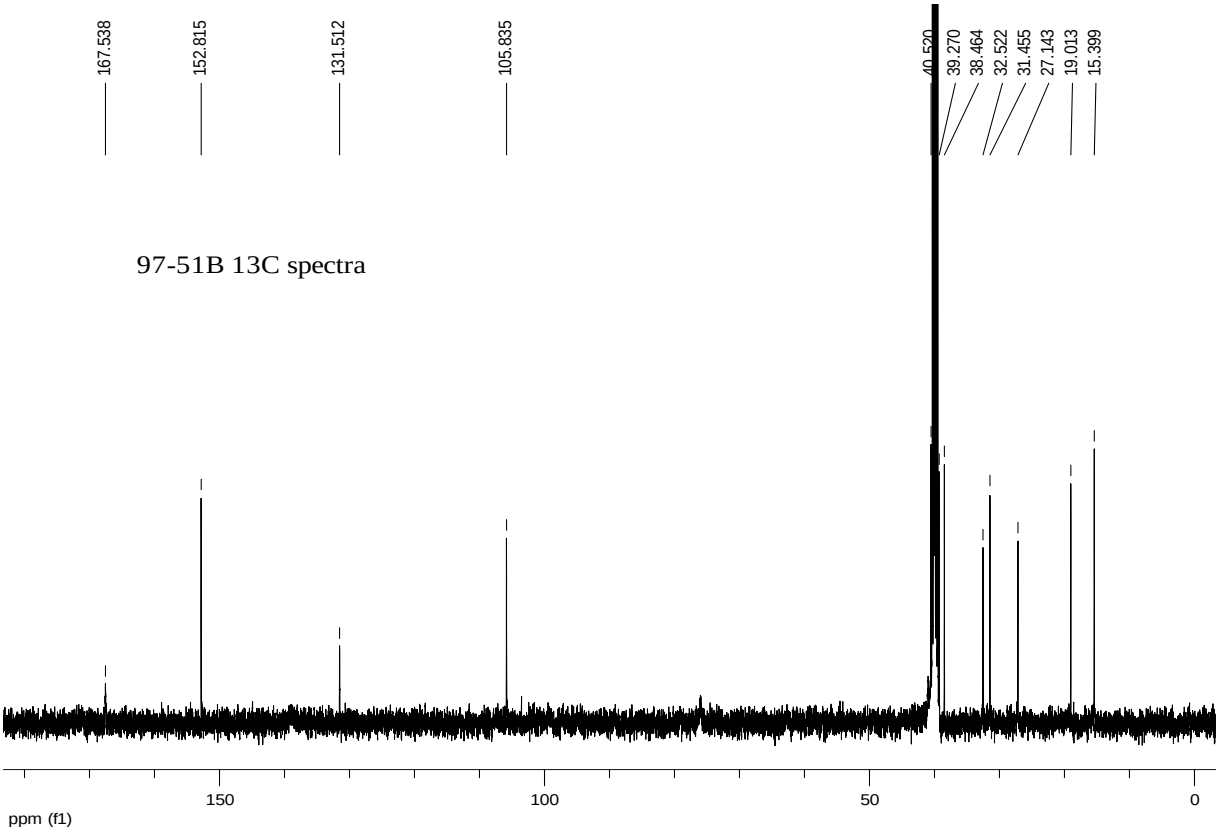
Appendix 11: ^{1}H and ^{13}C NMR of hentriacontane





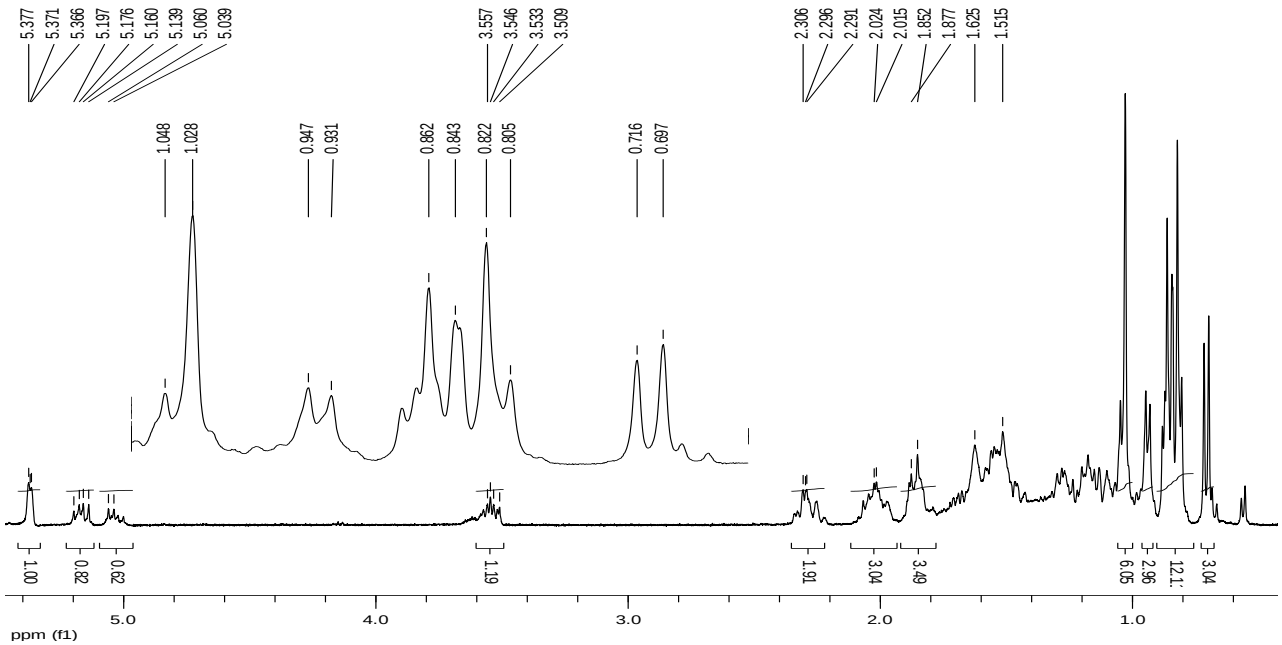
Appendix 12: NMR data of compound 120

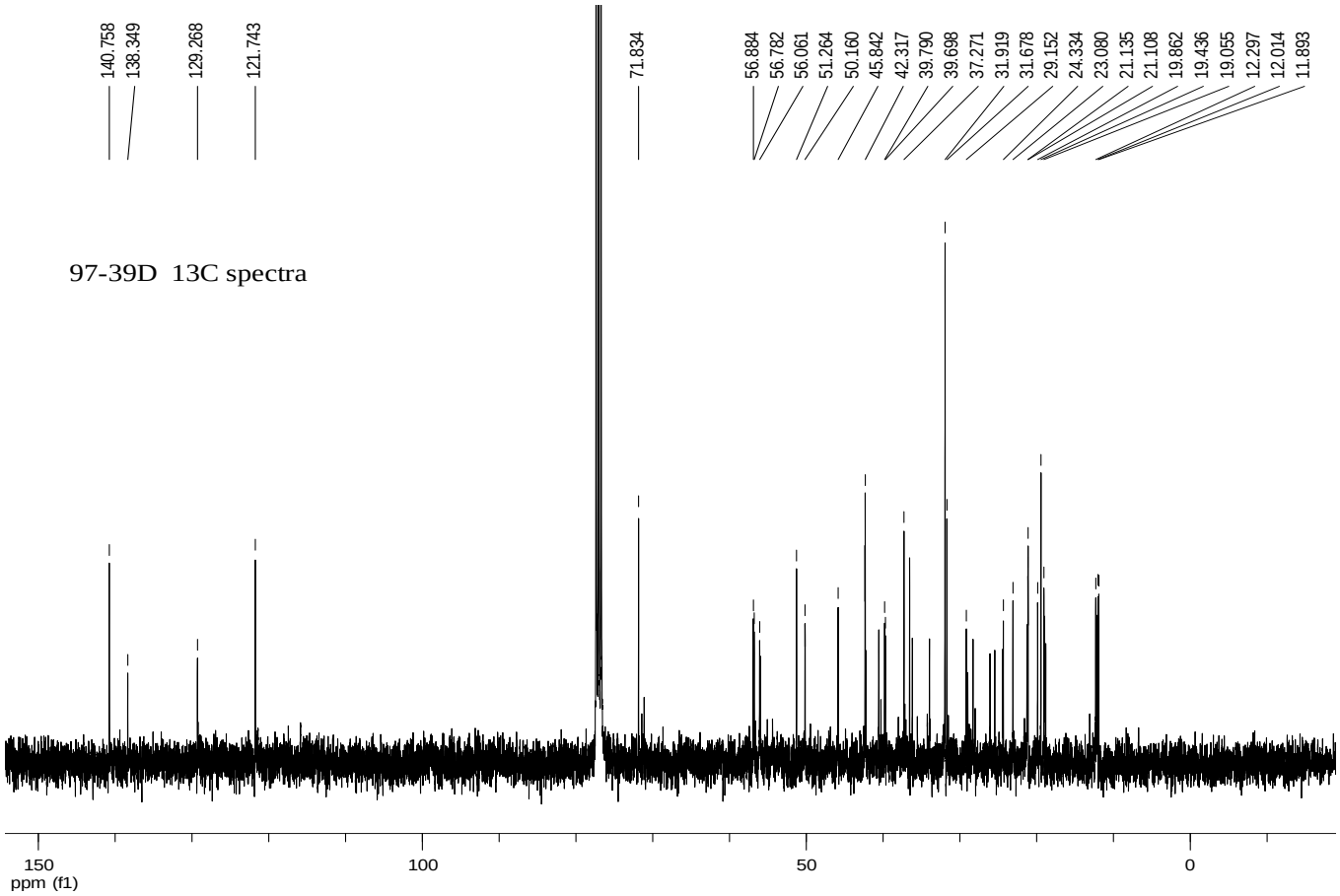




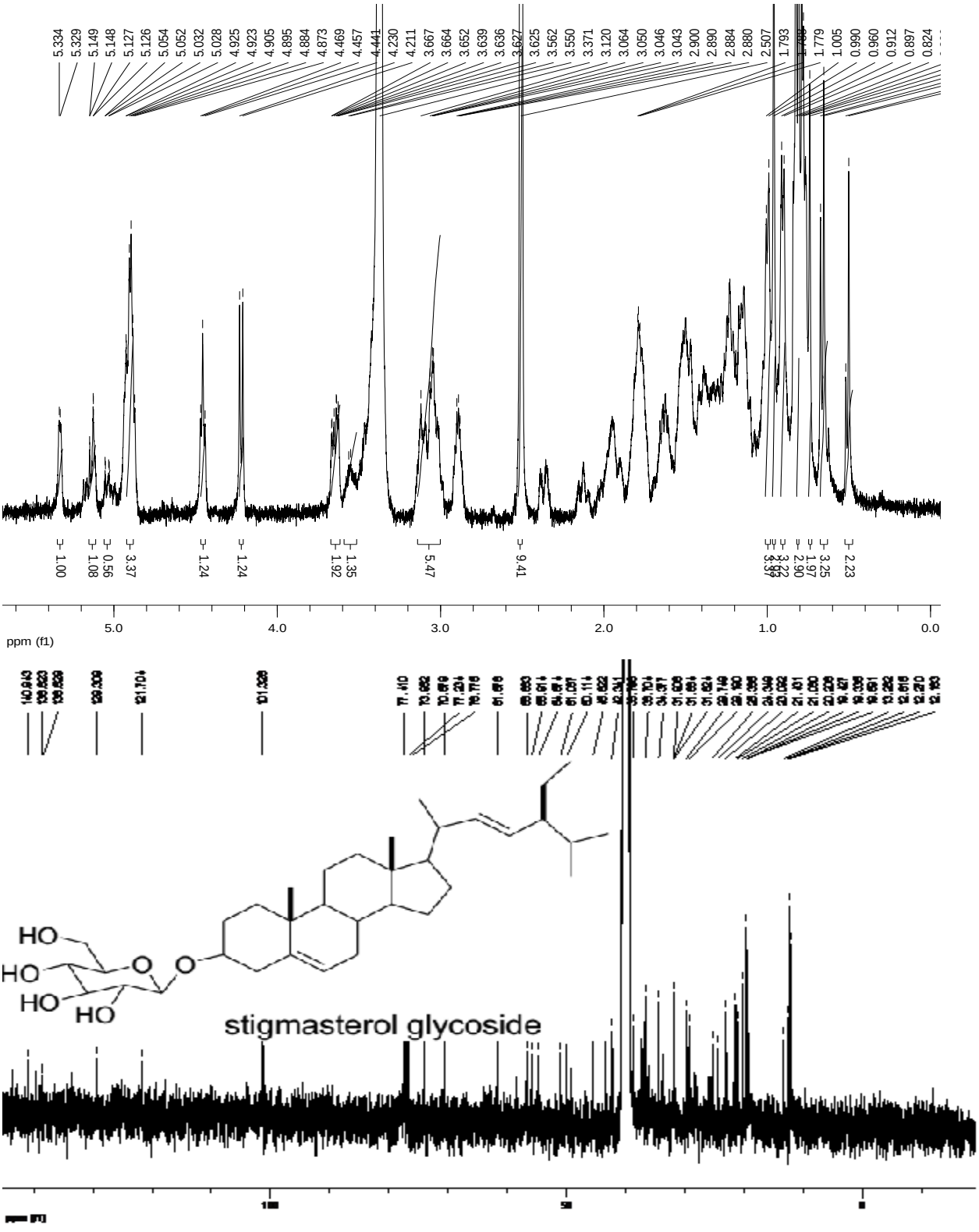
97-51B 13C spectra

Appendix 13: NMR spectra of stigmasterol (in $CDCl_3$)

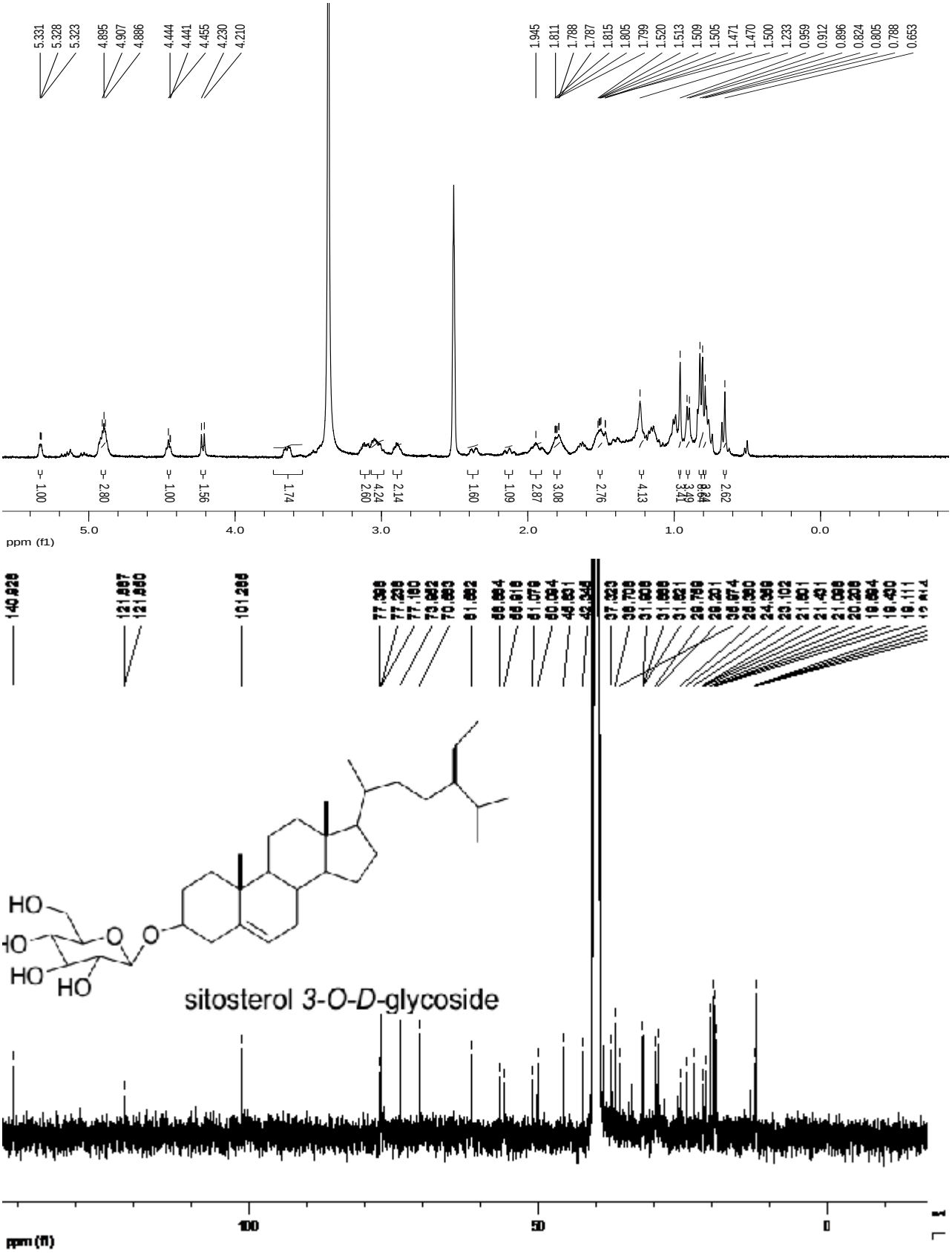




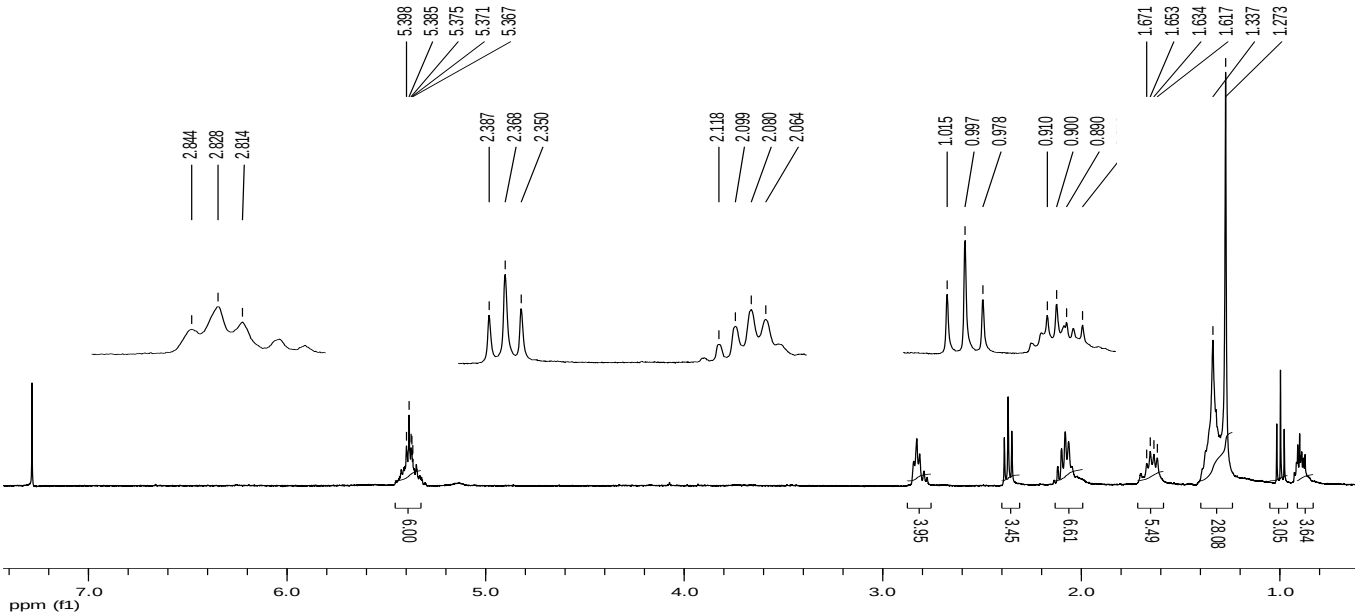
Appendix 14: ^{1}H and ^{13}C-NMR spectra of stigmasterol glycoside (DMSO)

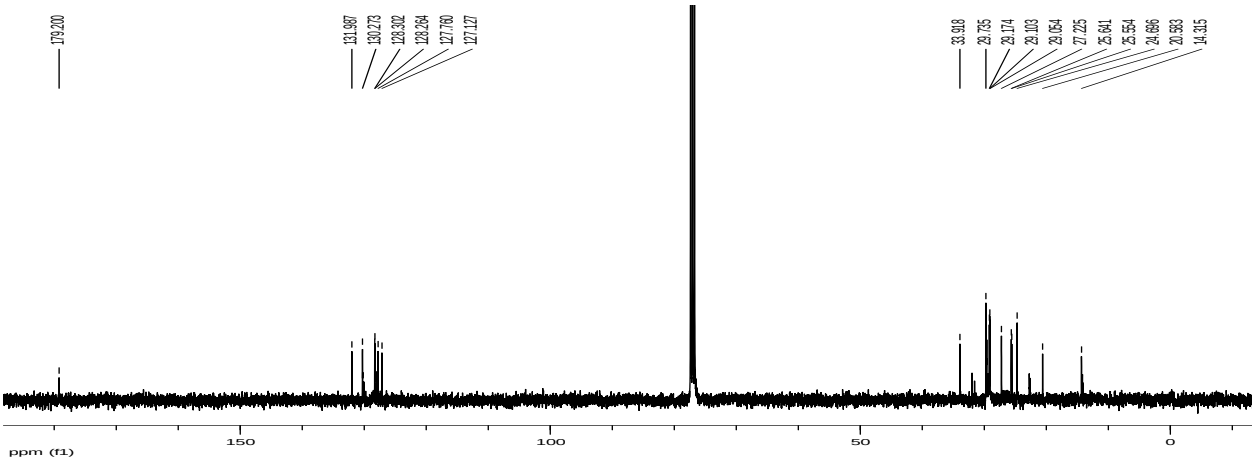


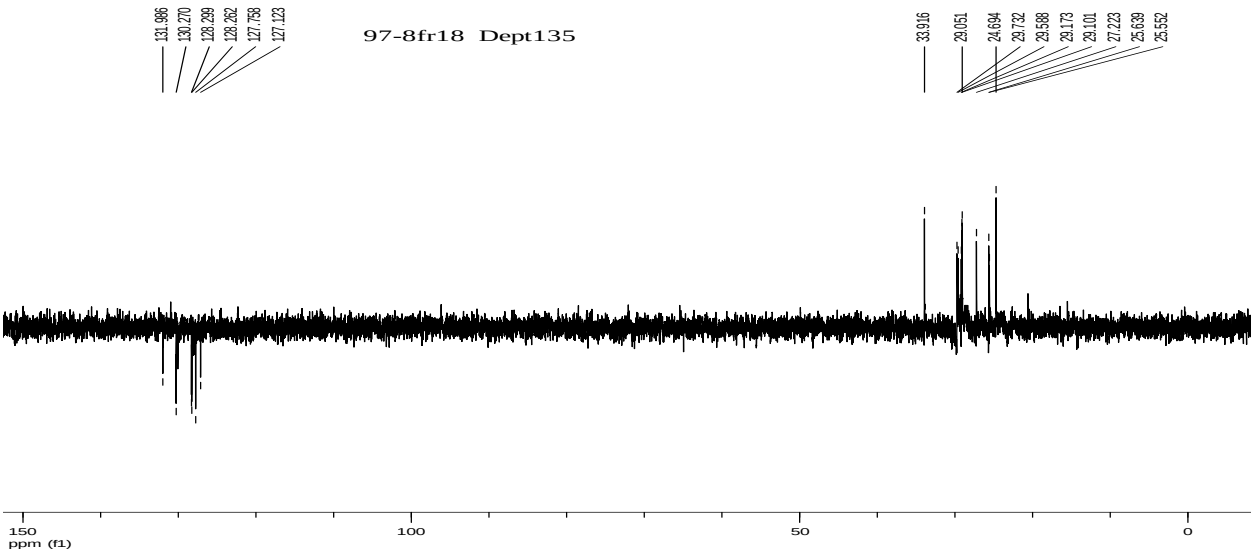
Appendix 15: NMR spectral data of sitosterol glycoside (DMSO)



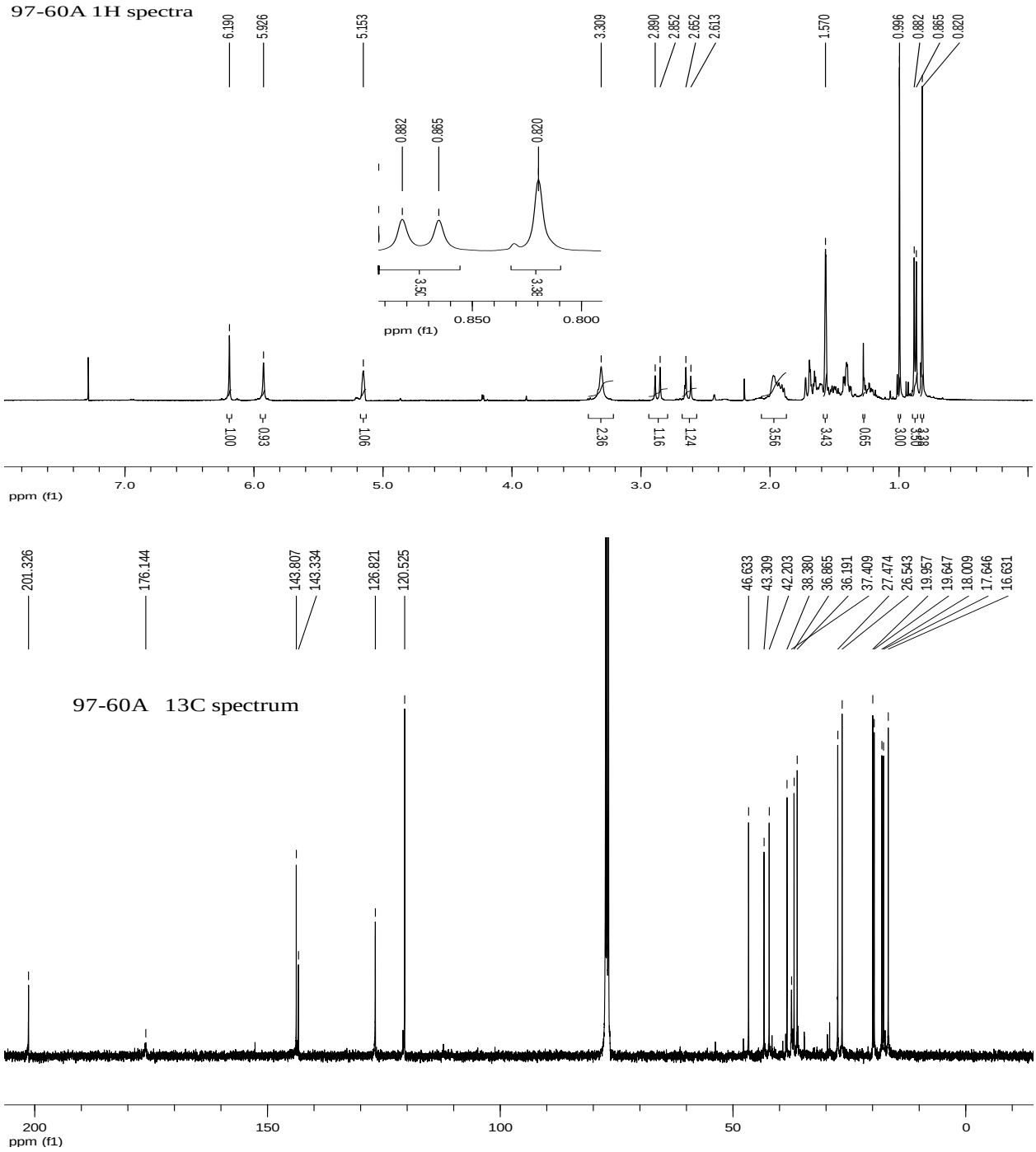
Appendix 16: NMR spectral data of Linolenic (9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic acid



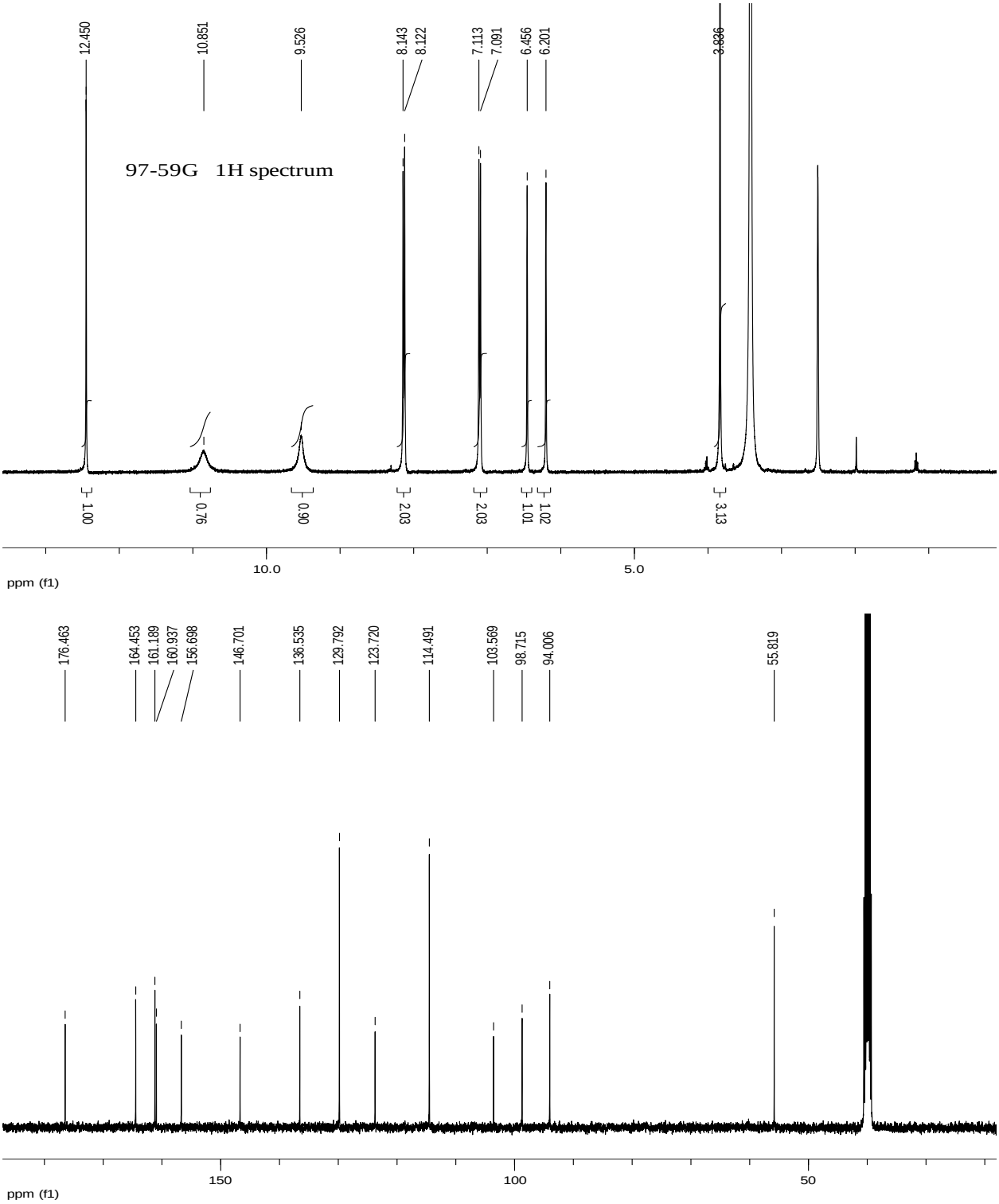




Appendix 17: NMR spectral data of diterpene from *P. schimperi* ($CDCl_3$)



Appendix 18: NMR spectral of kaempferide (DMSO)



Appendix 19: NMR spectral data of 1-heptacosanol ($CDCl_3$)