ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATIONS
ON
THE SEEDS OF VERNONIA GALAMENSIS

GRADUATE PROJECT CHEM. 774

BY: BELETE BEDEMO

JULY, 2006

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THE SEEDS OF VERNONIA GALAMENSIS

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SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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July 29, 2006

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GRADUATE PROJECT (Chem. 774)

DEPARTMENT OF CHEMISTRY

FACULITY OF SCIENCE

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ACKNOWLEDGMENTS

I would like to express my profound gratitude to my advisor Dr. Ashebir Fiseha for his consistent supervision and dedication in guiding my project work on this plant material. I am deeply grateful to my co-advisor Dr. Mekuria Tadesse* for his constructive encouragement and guidance while doing my project.

I would also like to acknowledge Bahir Dar University for giving me the opportunity to participate in postgraduate programme as well as EORC for providing plant material and giving me a chance to perform some part of the experiment.

My gratitude goes to Dr. Tesfaye Bekele*, Dr. Nigussie Alemayehu*, and Ato Fikermariam* for their encouragement of this work.

I am very much grateful to Dr. Wondmagegne Mammo. He helped me in running and processing 2D NMR spectra. I am indebeted to Dr. Yonas Chebude and w/t Medhanit Mammo for running IR spectra. I am also very much thankful to w/t Senait Dange for her help and share of laboratory experience in some technical problems.

I would also like to express my gratitude to w/o Adanech* and w/t Tadelech* for their help in preparing, cleaning and grinding of the seed material for extraction.

I am deeply grateful for the service of all NMR spectra and Ato Yoseph Atilaw for running the spectra.

My deepest gratitude also goes to my family and friends for their encouragement in my efforts to prepare this project.

Last but not least, I would like to express my deepest sense of gratitude to my lovely wife Yirgalem Gebre for her sisterly advice, help and continuous encouragement in preparing this project paper.

*Essential Oils Research Center

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List of symbols and abbreviations used

Co A------------------------------------Coenzyme A
DCM------------------------------------Dichloromethane
DEPT------------------------------------**D**istortionless **E**nhancment by **P**olarization **T**ransfer
FFA--------------------------------------Free Fatty Acid
GC --------------------------------------Gas Chromatography
HHPLC----------------------------------**H**igh **P**erformance **L**iqid **C**hromatography
Hz---------------------------------------Hertz
IR--InfraRed
m---multiplet
MS---------------------------------------Mass Spectroscopy
NMR-------------------------------------**N**uclear **M**agnetic **R**esonance
ppm--------------------------------------parts per million
Rf---retardation factor
t--triplet
TLC-------------------------------------Thin Layer Chromatography
UV--------------------------------------UltraViolet
VG--------------------------------------*Vernonia Galamensis*
J--a symbol representing coupling constant
δ(delta) ----------.----------------------the symbol used to indicate chemical shift value

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ABSTRACT

PHYTOCHEMICAL INVESTIGATIONS
ON
THE SEEDS OF VERNONIA GALAMENSIS

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The genus vernonia is one of the largest groups in the family Asteraceae and includes more than 1000 species distributed widely in tropical and sub tropical regions of Africa, Asia and America.

Vernonia galamensis is a new potential industrial oil seed crop for semiarid areas with very high content of naturally epoxidized vernolic acid (80% of the oil). The epoxy oil of this seed also contains linoleic acid (12% to14%), oleic acid (4% to 6 %), stearic acid (2% to 3%), palmitic acid (2% to 3%) and a trace amount of arachidic acid.

The dichloromethane and methanol extracts of the seed of vernonia galamensis afforded ***VG-3*** *and* ***VG-4A*** *respectively. The structural elucidation of these compounds was determined based on IR, UV, ^{1}H NMR, ^{13}C NMR and 2D NMR (COSY, HSQC and HMBC) spectra. Moreover, the literature values of selected ^{1}H NMR and ^{13}C NMR spectra of methyl vernolate are matched with ^{1}H NMR and ^{13}C NMR spectra of* ***VG-3*** *and* ***VG-4A***

1. INTRODUCTION

Recent decades have witnessed an increase in the rate of desertification of agricultural lands. More than one-third of our planet's land is now arid [1]. Water for crops and an increasingly human population is rapidly becoming a critically short commodity. Yet none of the major crop plants of the world is well adapted to arid lands [1]. On the other side, the development of industrial crops for semiarid zones is important in both developing and developed countries [2]. Hence, efforts have been made to introduce new crops, which are essentially noncompetitive with the existing crops and possibly provide a new and unique product for industrial use particularly for marginal rainfall areas. The species *Vernonia galamensis* (Cass.) Less., is a new industrial crop originating in Ethiopia, which combines all the possible merits for semiarid tropics and subtropics [1, 3].

The genus vernonia is one of the largest groups in the family Asteraceae. Chemical evidences especially the sesquiterpene lactones and flavonoids [4] support the hypothesis that the genus has two centers of origin one in Africa and the other in South America [5]. For instance, sesquiterpene lactones were isolated from more than 70 species of vernonia during chemical evaluation of the genus [6,7,8]. In addition, chromosome count provides information to distinguish the new and old world species [5]. Hence, new world species have chromosome count of n=17 and old world species n= 9,10 [5].

Several vernonia species have been found to be of various economic importances [5]. For instance two species of vernonia, *V. anthelmintica* and *V. galamensis* have been examined as potential sources of epoxy oils and vernolic acid [9]. A number of vernonia species have been widely used in traditional medicine and as food. Some others have also shown to contain active compounds, which possess cytotoxic, antitumer and antiviral activities [6].

In contrast, some vernonia species such as *V. baldwinil* and *V. cinerea* are trouble some as weeds in North America and Pan Tropical areas respectively [6].

2. BACKGROUNDS AND JUSTIFICATION

2.1. The genus vernonia

The genus vernonia includes more than 1000 species distributed widely in tropical and subtropical regions of Africa, Asia, and America and has two major centers of origin, South America and tropical Africa [5]. About 200 species ranging from annual herbs and shrubs to perennial trees are found in Africa of which about 50 species of vernonia have been recorded in Ethiopia (Tadessse, flora of Ethiopia, 4(1)).

V. galamensis (Figure 1) is known to naturally grow as weeds in fields or in wood lands under a wide range of agro-ecological conditions of Africa [3,5]. It is a break crop to prevent pest damage, an alternative crop for farmers and plays a great role in oleo chemical industry. Developing countries can also capitalize on growing it for export or for their own industrial development. It is originally found in Ethiopia, most suitable for dry land areas, essentially noncompetitive with the existing crops and high cash crop as primary source of income for farmers.

The subspecies galamensis is the most widely distributed, highly diverse and has four botanical varieties, namely varity galamensis, varity petitiana, varity australis and varity ethiopia [1,2]. It grows in areas with as little as 20cm of seasonal rainfall [10]. A porous, well-drained and sandy soil is best for its growth. Vernonia plant does not dowel on heavy clay. Pest survey of *V. galasmensis* showed that a number of insects and diseases have been recorded [1]. There is also a serious problem of vernonia rust disease on the leaves and pods of the plant at Wendo Genet, in Ethiopia. A superior species of vernonia in terms of oil and vernolic acid content was originally found in Ethiopia [11].

Figure 1. *Vernonia galamensis* plants

2.2. Vernonia oil and its composition

Vernonia galamensis is a new potential industrial oil seed crop for semi-arid areas of the tropics and subtropics, with very high content of vernolic acid [2,3,7,12,13]. The vernolic acid is primarily present as the triglyceride, trivernolin (Figure 2) [2, 14].

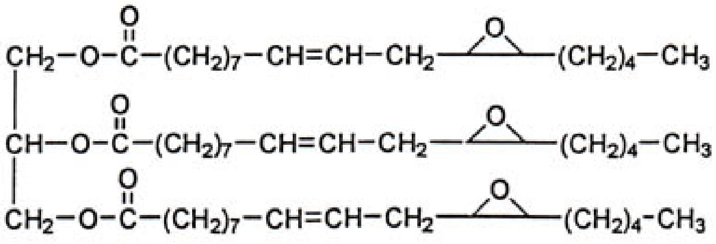


Figure 2. Structure of trivernolin

The seeds of this plant contain 40% epoxy oil, which when hydrolyzed, yields different fatty acids with variable composition as detected by GC.

The fatty acid profile of V. galamensis oil as reported by Ayorinde et al. 1990 is: vernolic acid (79% to 80%), linoleic acid (C18:2) 11% to 12%, oleic acid (C18:1) 4% to 6%, stearic acid (C18:0) 2% to 3%, palmitic acid (C16:0) 2% to 4%). Thompson et al., (1994) also reported the fatty acid composition of vernonia oil as follow: vernolic acid (72% to 82%), linoleic acid (12% to 14%), oleic acid (4% to 6%), stearic acid (2% to 3%), palmitic acid (2% to 3%), and a trace amount of arachidic acid (Figure 3) [1,7,12,15].

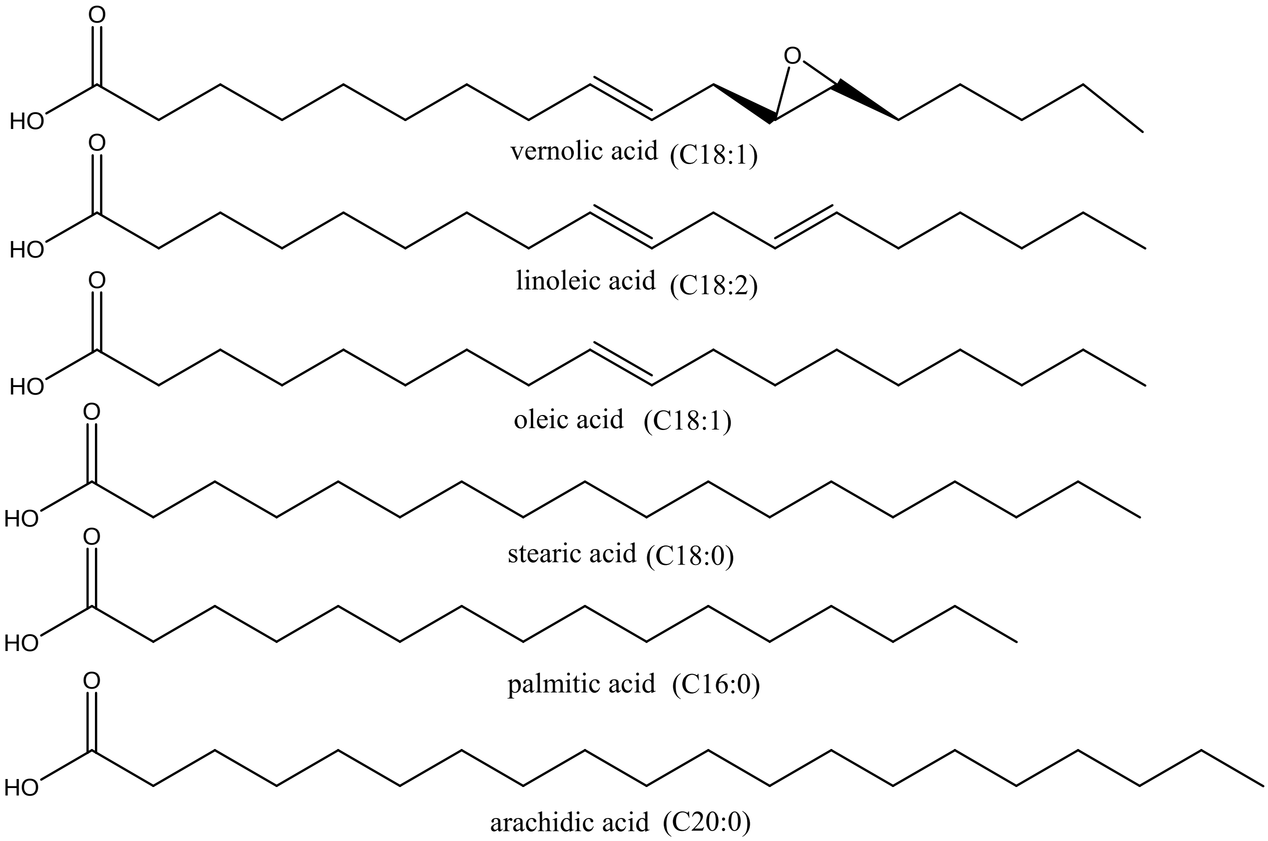


Figure 3. Structures of fatty acids of vernonia oil [16]

2.3. Economic importance and reactivity of the oil

The development of industrial crop for semiarid zones is important in both developing and developed countries. Many plants suitable for arid and semiarid zones are regarded as having high potential as industrial crops [2]. *V. galamensis* is an oil seed crop and as a candidate crop for arid and semiarid zones in both developing and developed countries [2].

Chemically, vernonia oil is similar to epoxidized soybean and linseed oils. Soybean oil contains only 50% of linoleic acid as its principal fatty acid and linseed oil contains just 57% of linoleic acid as its principal fatty acid, where as vernonia oil is rich in a single fatty acid known as vernolic acid. This makes it an especially attractive raw material for industry. In addition, vernonia oil has several unique properties: it is a transparent homogenous liquid at room temperature with a low viscosity of about 110 cps, and it is even pourable at room temperatures below 0^0C. In contrast, epoxidized soybean oil and epoxidized linseed oils are highly viscous, with a viscosity of about 300-1500 cps; they are semi-solid at 10^0C and are non pourable below 0^0C[2].

Nowadays there is a large industrial market for synthetically epoxidized vegetable oils such as linseed or soybean oils, but the epoxidation process is expensive and has a lot of environmental problems. Vernonia oil, on the other hand, is already epoxidized in nature by enzymatic action, and may be able to fill some of those market niches or could be used as a substitute for currently used epoxy oils [15].

The presence of epoxy group, the low viscosity and polymerizing characteristics of this oil makes it especially valuable as a solvent in industrial coatings and paints [17]. Some of the products that are being developed from vernonia oil are also degradable lubricants and lubricant additives, epoxy resins, plastic formulations of polyvinyl chloride, adhesives, insecticides and insect repellants and reactive monomers in polymer synthesis [17, 18]. Other applications of the products that developed from vernonia oil are for the construction of polyurethane foams, for the synthesis of interpenetrating polymer networks, as PH stabilizers, waxes, glues, emulsifiers and rust suppression, and in organic formulation of carriers for slow-release pesticides and herbicides.

Vernonia oil has also been used as a source of hydroxyl alkoxy fatty esters and for the synthesis of epoxy secondary amides [2]. Current vernonia product on the market includes Vernola super Gloss, a car-care product used on tires, Vinyls, flash boards, leather, and rubber bumpers (Cunningham, 1997).

The unique and special structure of epoxy acid within the triglyceride enables a wide variety of reaction characteristics of the ester group, the double bond, and the epoxy group to occur as shown below (Figure 4) [2].

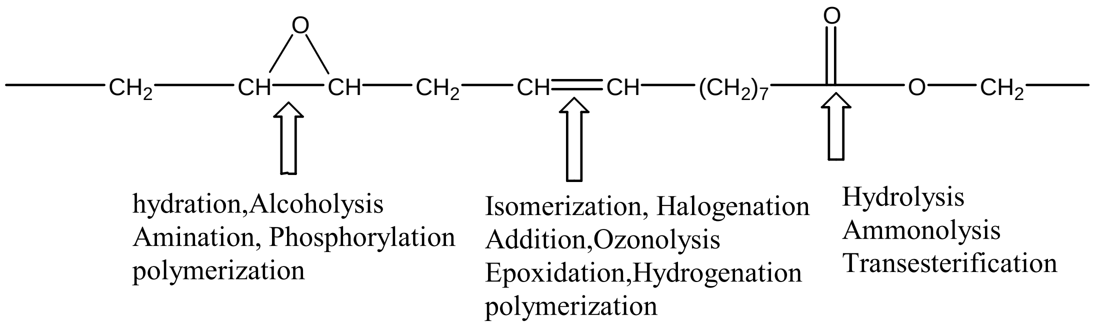


Fig 4. A wide variety of reaction characteristics of epoxy acid within the triglyceride

Several synthetic routes for the production of selected special chemicals, in which the vernolic acid group serves as a key starting material, have also been explored [19].

For instance bombykol (**7**) (which has been identified as a sex pheromone of the silk worm Bombyx mori and can be used in pest control) is synthesized from vernolic acid (Figure 5) [19].

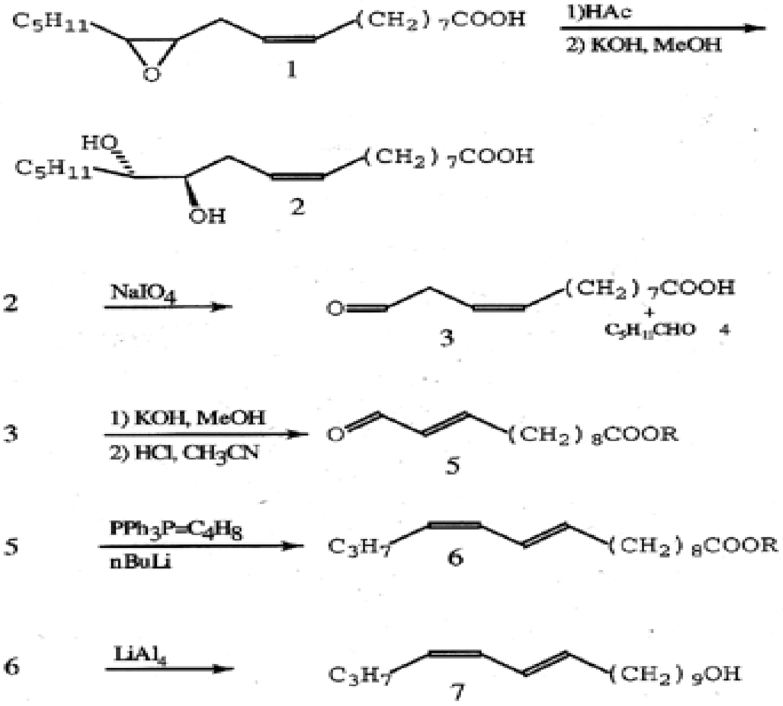


Fig 5. Synthetic route from vernolic acid to bombykol

In addition, traumatic acid (**9**) (which is active as wound hormone of plants and can be used as an intermediate in prostaglandin synthesis) is synthesized from vernolic acid-derived intermediate (Figure 6) [19].

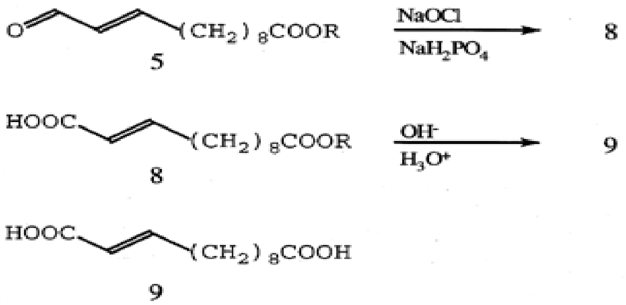


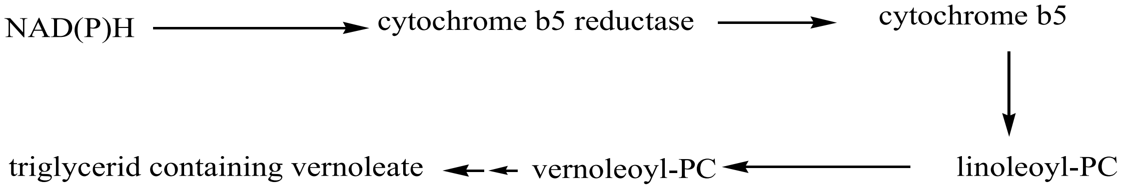
Fig 6. Synthesis of traumatic acid from the vernolic acid-derived intermediate 12-oxo-10-E-dodecenoic acid.

2.4. Biosynthesis of vernolic acid

Much of the researches concerning unusual fatty acids has been focused on the identification, amount and composition of fatty acids found in various plant species and less is known about the mechanism responsible for the biosynthesis path way and accumulation of unusual fatty acids.

V. galamensis accumulates vernolic acid in seed triglyceride, not in any other lipid class or plant part. The biosynthesis of vernolic acid has been partially characterized and reported by Bafor et al. in 1993. It is known to be synthesized from linoleic acid (18:2) by an enzyme epoxygenase in endoplasmic reticulum in one step reaction. The substrate for developing vernonia seed epoxygenase appears to be linoleoyl-phosphatidyl choline (PC). That is the epoxy group of vernolic acid has been shown to result from the insertion of an oxygen atom at the Δ^{12} double bond of linoleic acid bound to phosphatidyl choline (PC) in seed of *V. galamensis*. Either NADH or NADPH is necessary for activity and they support the activity similarly. The activity of this enzyme is inhibited by CO and cyanide.

Therefore a likely path way for the redox activity for the epoxygenase reaction is:



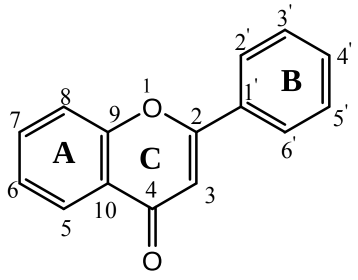
2.5. Secondary metabolites

These are in principle, non-essential to life but they definitely contribute to the species’ fitness of survival. They are more characteristics for the particular biological group, such as family or genus, and apparently the synthetic machinery involved here is related to the mechanism of evolution of species. They are of restricted occurrence and of no apparent utility. These include: phenolics, terpenoids and steroids, and alkaloids [16,20].

A survey of literatures on vernonia showed that most of the chemical investigations on the genus had taken place over the last two decades. However, out of the 1000 species, less than 150 have been thoroughly examined [6]. Chemical evidences indicated that the secondary metabolites that were isolated from the genus vernonia include: flavonoids, terpenoids and sterols, acetylenes and vernolic acid. But the more frequent compounds are falvonoids and sesquiterpene lactones [4]. In addition, vernolic acid is one of the principal fatty acid of *V. galamensis* and *V. anthelmintica* [9].

2.5.1. Flavonoids

Flavonoids constitute of one of the largest groups of naturally occurring phenolic compounds [16, 20,21]. They have two benzene rings connected by three carbon units which may or may not from a ring [22,23]. The rings are labeled as A, B and C and the carbon atoms are numbered as shown in (**10**) and (**11**). That is, flavonoids contain fifteen carbon atoms in their basic nucleus which are arranged in a C_6-C_3-C_6 manner [22]. They are generally water soluble and the more conjugated compounds often are bright yellow [16].



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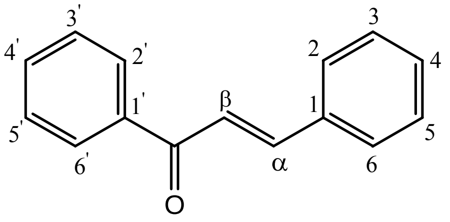

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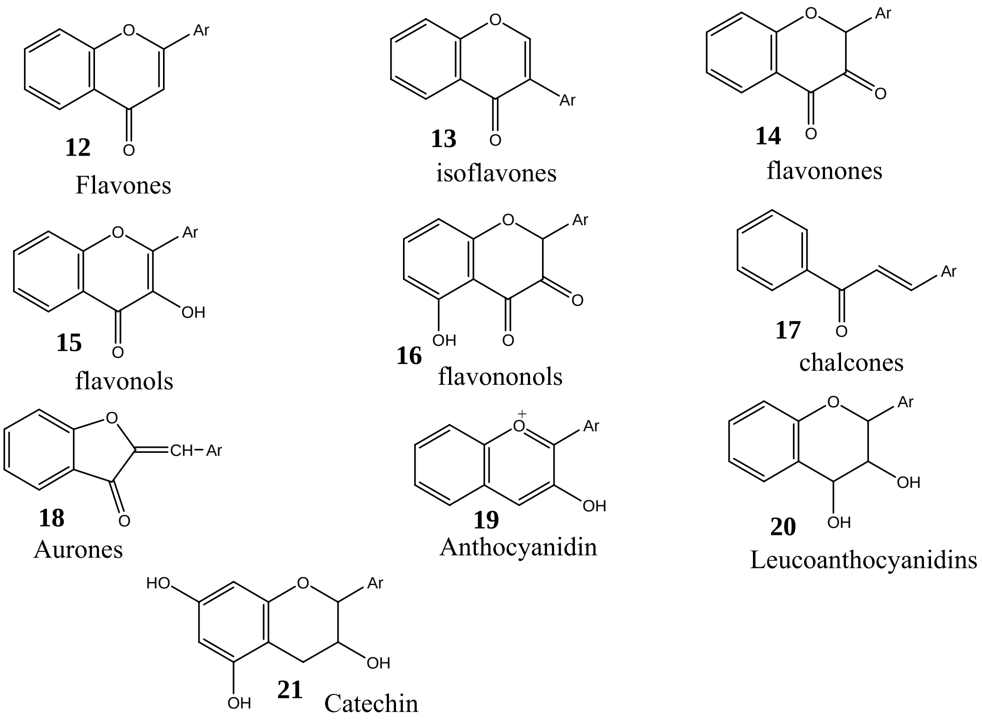
2.5.1.1. Structural variation and classification

Flavonoids exist in plants as aglycones (flavonoids with out attached sugars), glycosides, sulfates and biflavonoids [20,22]. The oxidation of the C-3 fragment (ring C) and the size of this ring is the basis for classification of flavonoids in to the major classes [16,20,22].



12 Flavones

13 isoflavones

14 flavonones

15 flavonols

16 flavononols

17 chalcones

18 Aurones

19 Anthocyanidin

20 Leucoanthocyanidins

21 Catechin

Figure 7. Structures of some common classes of flavonoids

That is, different classes with in the group are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups. These include the flavones, isoflavones, flavonones, flavonols, flavanonols, chalcones, aurones, anthocyanidins, Leucoanthocyanidines and Latechins (Figure 7) [16].

2.5.1. 2. Distribution and biological activities of flavonoids

Phenolic compounds are found through out the plant kingdom, but the type of compounds present varies considerably according to which plant group is under consideration [20,24]. Their occurrence is restricted to higher plants and ferns.[22,26 Mosses contain a few flavonoid types but they are absent in algae, fungi and bacteria [20,24]. Flavonoids appear in all vascular plants but some classes are more widely distributed than others; while flavones and flavonols are universal, isoflavones and biflavones are found in only a few plant families [16,20].

The flavanones and flavanonols are fairly rare and normally exist as their glycosides. The anthocyanidins are the common red and rare blue pigments of flower petals and can make up as much as 30% of the dry weight of some flowers [16]. They exist typically as glycosides. The chalcone is more fully conjugated and normally brightly colored. The aurones are golden yellow pigments common in certain flowers [16].

Biologically, flavonoids play a major role in relation to insects pollinating or feeding on plants, but some flavonoids have a bitter taste, repelling certain caterpillars from feeding on leaves [20]. In addition, some known biological activities of flavonoids include activities such as nector guide components, anti inflammatory and anti-oedima, cardio vascular, inhibition of aldose reductase, phytoalexins, antimicrobial and antifungal [22,25-31].

2.5.1. 3. Biosynthesis and biogenic relationships

All classes of flavonoids are biogenetically related by a common biosynthetic path way which in corporates precursors from both the “shikmate” and the “acetate-malonate” pathways (Figure 8) [22,24].

The C_6–C_3 unit of rings B and C, for which phenylalanine is the precursor, is derived from the shikmate path way [16,20,31]. The C_6 unit of ring A, is formed from the head-to tail condensation of acetyl Co A, the precursor of the acetate–malonate path way [35]. The basic flavonoid biosynthetic precursor (the first intermediate) thought to be formed immediately after the confluence of the two pathways is a chalcone.

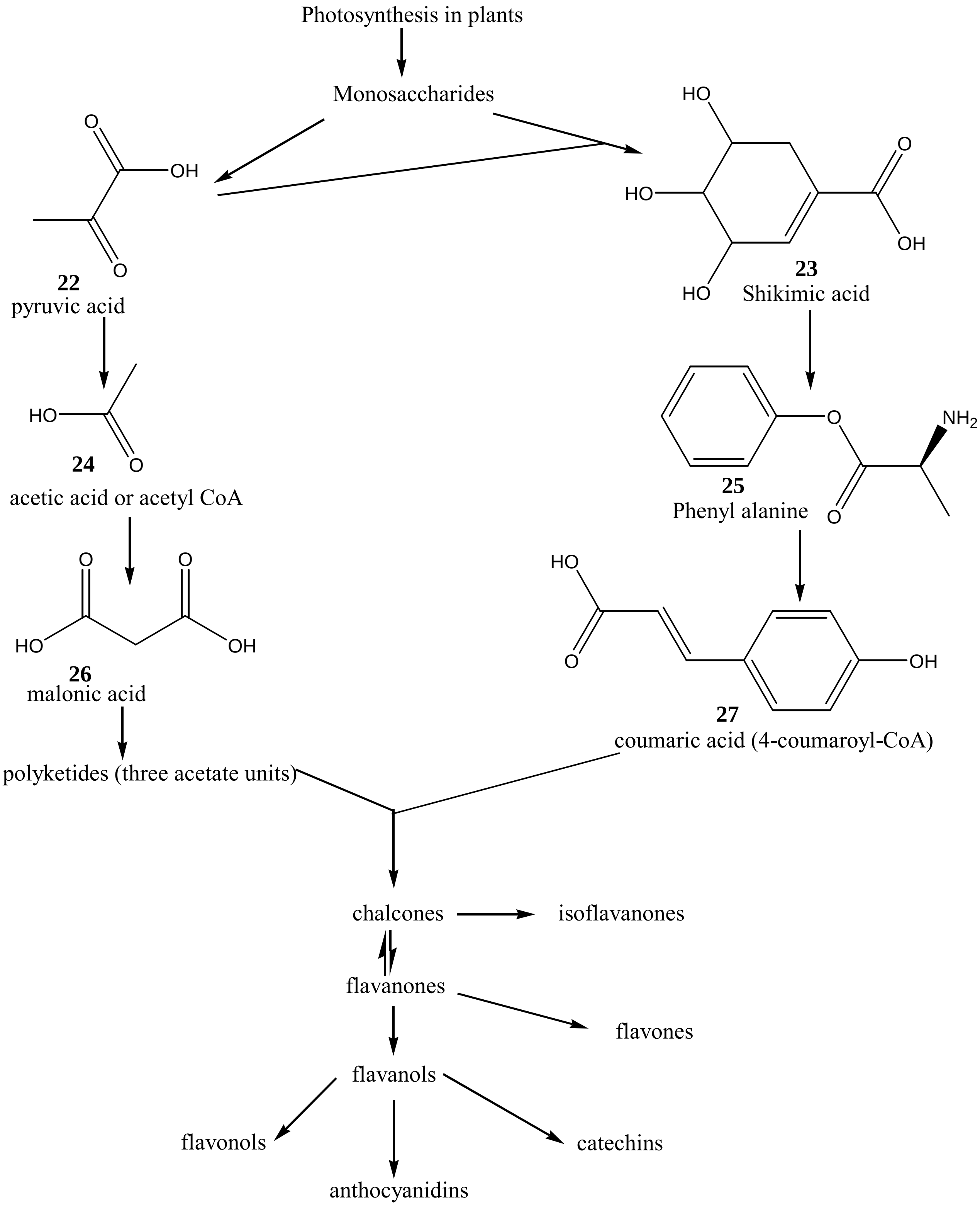


Fig 8: Biosynthesis and biogenetic relationships of flavonoids

2.5.1. 4. Identification

Flavonoids change color when treated with base or with NH_3; thus they are easily detected on chromatograms or in solution [24]. For example, methanolic KOH spray gives a yellow spot on silical gel plates and relation between the spot appearance and the flavonoid structure can be made. Their color reactions offer only a broad identification of the flavonoid type because with in any given class, the color produced depends on the oxidation pattern.

In addition to the above color changes, equally informative date can be obtained form spectral characteristics. Structural elucidation of flavonoids and other natural products are well served by the application of UV, IR, NMR and Ms spectroscopic techniques.

UV absorption spectroscopy is one of the most useful techniques for flavonoid structural analysis. The flavonoid UV spectrum consists of two absorption maxima in the region 240-550 nm. These are referred to as band I (300-550 nm) and band II (240-285 nm) [22,24].

The position and intensities of the maxima give valuable in formation about the nature of flavonoid and its oxygenation pattern [32]. Changes in A ring substitution are reflected in band II absorption while changes in substitution of B and C- rings are reflected in band I absorption.

IR, ^{1}H-NMR, ^{13}C-NMR and Ms Spectroscopes are also essential tools used for the structural elucidation of flavonoids.

2.5.1. 5. Flavonoids of the genus vernonia.

Most of the flavonoids isolated from the genus vernonia are based on the flavones (apigemin and luteolin types) and the flavonols (kaempferol and quercetin types) [33-36]. The compounds include the aglycones, their methylated derivatives and thier O-and C-glycosides. Exceptions include the falvanone hesperitin isolated from *V. brevitolia*, two new flavanones (hesperidin and homoespertin) isolated from *V. diffusa* and tricin isolated from *V. remotiflora* [4,37]. Flavonoids have been used in assisting the classification of the genus It appears that a complex flavonoid profile might represent a primitive character with in the genus [22].

It has been observed that species which are recognized as more advanced on other than chemical grounds (eg. morphology) contain a relatively small number of flavonoids. A large number of flavonoids appear as primitive character. This reduction trend in evolution has been observed in several cases [5,27,33].

In addition, new world species show the most complex falvonoid patterns while old world species show the simplest flavonoid pattern. Old world species give only flavones while the new world species give either flavones or flavonols, or both [27,33].

2.5.2. Terpenoids

Terpeniods are among the widespread and chemically diverse groups of natural products (secondary metabolites) which are found in all parts (that is seed, flower, foliage, roots) of higher plants and in fungi, marine organisms and insects [16,20,38]. Fortunately despite their structural diversity, they have a simple unifying feature by which they are defined and may be easily classified [16]. Their carbon skeleton is built up from the union of two or more of the isopentyl (isoprene) units which are usually linked in a head- to- tail manner, with more notable exceptions. Terpenes are classified by the number of five carbon units they contain: hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25} or very rare), triterpenes (C_{30}) and tetraterpenes(C_{40}) [16,20,31,38].

The sesquiterpenoids provide a remarkable diversity of the carbon skeleton compared to the other species and are the most important class of terpenoids in the Asteraceae family [20,39]. Monoterpenes and sesquetepenes are also major components of essential oils [16].

Studies of terpenoids have been stimulated by their wide range of biological activities such as allergenic reagents, cytotoxic and antitumor agents, regulators of plant growth, antischistomal agents, antimicrobial agents and accessory pigments in photosynthesis [16,20,38-443].

Terpenoids have a common biosynthetic origin based on the mevalonic acid derivate isopentnyl pyrophosphate. This is formed from acetyl CoA Via mevalenic acid (Figure 9) [16,20,31,38,43].

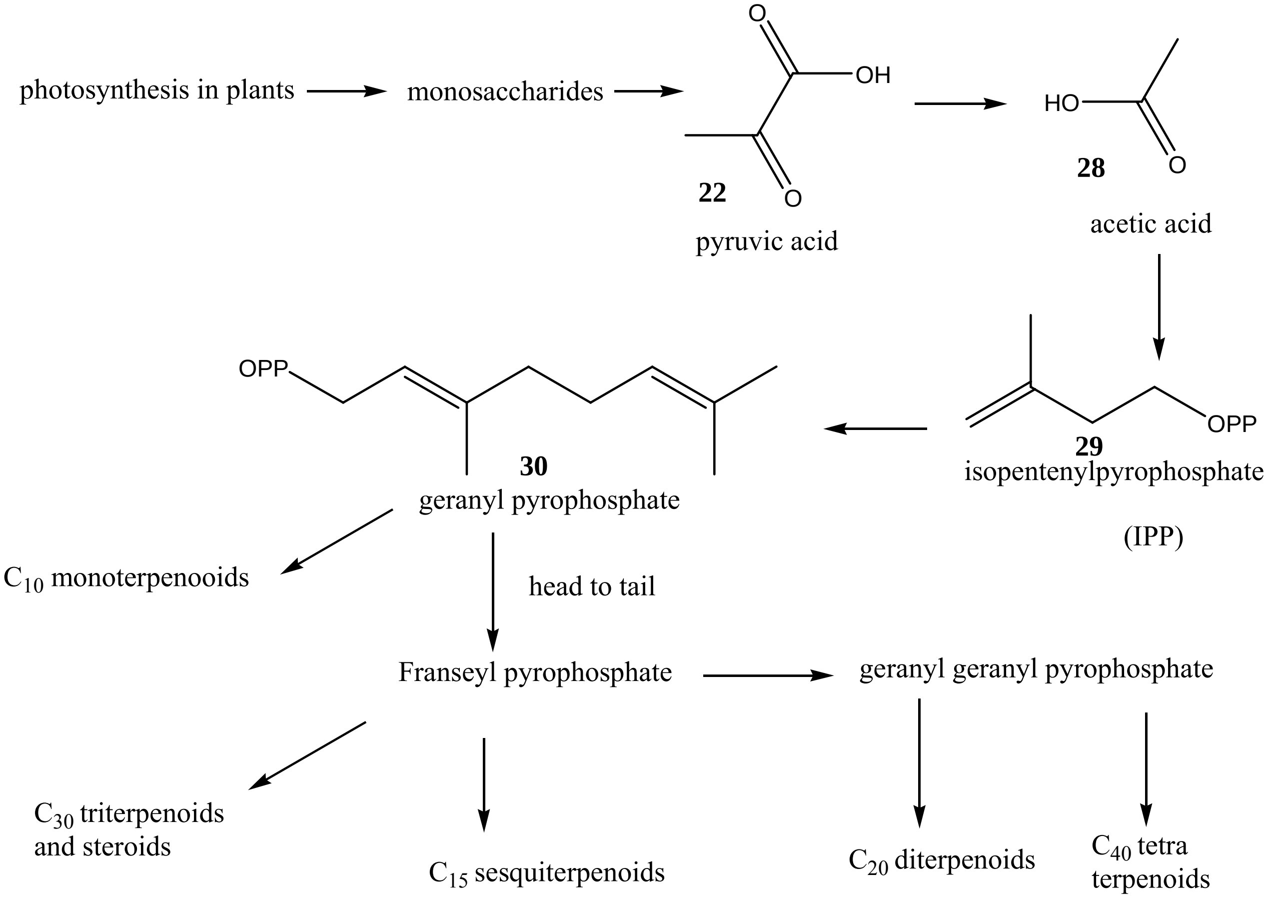


Figure 9. Biosynthesis and biogenic relationships of terpenoids.

Detection of terpenoids is usually difficult since all of them are colorless except carotenoids [42]. There is no universal chromatographic reagents specific for terpenoids. Detection is usually carried out by spraying sulfuric acid or vanillin /sulfuric acids on T L C plates and heating. Structural elucidation can be carried out using spectroscopic tetchiness, namely IR, ^{1}HNMR, ^{13}CNMR and Mass Spectra together with biogenetic considerations.

2.5.2.1. Sesquiterpene lactones

There has been a dramatic increase in the number of reports dealing with isolation and structural elucidation of sesquiterpene lactones over the last two decades. This increased interest has been due to their successful use as taxonomic markers and their various biological activities [44]. They are found to occur in plants and fungi [39]. They are also the major constituents of the tribe

Vernonieae and the genus vernonia. Reports indicated that the number of sesquiterpene lactones isolated from the tribe Vernonieae goes up to 236 [45].

2.5.2. 2. Sesquiterpene lactones of the genus vernonia

Sesquiterpene lactones are widely distributed in Asteraceae family. Germacranolide type lactones are the largest class of sesquiterpene lactones found in the genus vernonia. Glancolide-type lactones which contain an endocyclic α, β-unsaturated lactone in which C-13 is oxidized and usually bears an acetate group are also characteristics of this genus [46]. Among the new world species examined so far, there are many reports of glaucolides and hirsutinolides [46,47]. In addition to eudesmanolides, guaianlides and elemanolides, both glaucolides and non-glaucolide type germacranolides have been reported from the old world species [46]. Although sesquiterpene lactones of the new world species had been reported to be sufficiently different from those of the old world species,[27,33] investigation of several south African species have shown a close relation to the American species [48-51]. Species from Malawi however gave sufficiently different sesquiterpene lactones [52].

Generally, the big diversity in the genus vernonia and the pronounced difference in the chemistry of different species may be explained by the degree of evolution in the genus [53].

1.1. Objectives of the project

Although very little chemical investigation had been carried out on the vernonia of Ethiopia, series of studies on *V. galamensis* are currently under way. The potential use of its epoxy oil in many industries, the higher oil content, its tolerance to drought and its unique character compared to other oil seed crops led us to study this plant.

This project focuses on phytochemical investigations on *V. galamensis* sub spp. galamensis var.ethiopica. The objective is to determine the oil content, measure physicochemical parameters of the oil and to undertake chemical characterization of *V. galamensis* for its chemical constituents especially the secondary metabolites using different spectroscopic techniques.

1.2. Methods and Procedures

The established method of seed oil analysis involves basic hydrolysis of the crude oil followed by acidification and methylation with an appropriate reagent to obtain methyl esters. The methylated oil sample is subsequently analyzed by gas chromatography for fatty acid methyl esters. But in this project gas chromatographic technique was not carried out due to time constraint.

In the first part of the experiment, the crude vernonia oil was extracted with n-hexane using Soxtect1040 and Soxhelet extraction systems. Oil refining process was conducted to remove variable amounts of impurities and physico chemical characteristics of the refined oil were then determined.

Solvent extraction with dichloromethane and methanol were carried out to isolate secondary metabolites. TLC and column chromatographic techniques were conducted as detailed in the experimental part. The pure samples were then taken for spectral determination.

3. RESULTS AND DISCUSSIONS

3.1. Oil yield content

The seed of vernonia galamensis has been examined as potential source of epoxy oil [9]. The oil yield of vernonia seed is influenced by various parameters, the most being the type of the seed (ecotype), the solvent used, the particle size of the extracted materials (the fineness and coarseness of the powder) , and the time of extraction. The oil content is differing due to the difference in the degree of grinding. The fine powder gives better results than the coarse. This may be attributed that fine powder is more exposed towards the solvent and the solvent is more effective in leaching the oils [58].

Table 1: The Oil yield content of of *V. galamensis* accessions under current investigation

Sample code	Ave. sample wt.(g)	Ave. moisture content %	Ave. wt.of oil(g)	Aver. Oleoresin yield % fresh based	Aver. Oleoresin yield% dry based
v-1	11.673	5.147	2.626	22.473	23.692
v-2	12.421	4.481	3.171	25.532	26.729
v-3	12.227	4.973	2.575	21.055	22.157
v-4	12.394	4.673	2.636	21.262	22.304
v-5	11.738	4.424	2.626	22.376	23.411

Significant variations in oil and vernolic acid contents among accessions were found. Oil content ranged from13.8% to 53.7% with a mean of 24% and vernolic acid content ranged from 40% to 75% with a mean of 62.4% was reported [10].

Evaluation of five accessions of *V. galamensis* seeds collected from Dilla and Ziway showed variations in oil content and oleoresin yield% among them (Table 1). From this data we can conclude that the oil content and oleoresin yield% is higher for V-2 (the sample collected from hot region of Dilla) and lowest for V-3 (the seed material collected from Bulbula and Meki regions around Ziway). This variation in oil content or oleoresin yield% could be due to ecological factors such as soil type, rain fall and the particle size of the extracted material.

Comparing the oil content reported in the literature above with the values in the current investigation showed good agreement. But in this investigation there is no significant variation among the accessions because the seed materials were collected from the areas with insignificant variations in environmental conditions.

3.2. Physico-chemical characteristics of the oil

The physico-chemical properties that were measured for vernonia oil are refractive index and specific gravity. In contrast to soybean and linseed oils with high viscosity (300-1500 cps), vernonia oil has low viscosity (110 cps); and is pourable even below 32^0F [11]. This low viscosity makes vernonia oil an attractive choice as a raw material in many oleochemical industries.

Table 2. Physico-chemical characteristics of the vernonia oils obtained from different accessions.

Sample code	Ave.specific gravity	Ave.refractive index
v-1	0.976	1.4790
v-2	0.924	1.4720
v-3	0.926	1.4723
v-4	0.978	1.4745
v-5	0.944	1.4764

Examination of physico-chemical characteristics of refined vernonia oil of five accessions is presented (Table 2). The significance of measuring these parameters is to determine the quality of the oil either by comparing with the previously reported data of the same sample or other related samples. There is no significant variation in physicochemical characteristic values of vernonia oils obtained from different accessions.

3.3. Isolation and Characterization

Phytochemical investigation, in the course of this project, of seeds of *V. galamensis* has resulted in the isolation of one compound from dichloromethane extract and one compound from methanol extract. Characterization of the isolated compounds will be explained as follow.

3.3.1. Characterization of VG-3 (Compound 31)

This compound was isolated from dichloromethane extract of the seed material by repeated column chromatography using n-hexane-dichloromethane (98:2) solvent system. Characterization was determined by using spectroscopic techniques.

The UV spectrum (Appendix 1) absorption band at λ=277 nm indicates the presence of chromophore. In the IR spectrum of **VG-3** (Appendix 2) absorption bands at 3010 cm^{-1}, 2928 cm^{-1}, and 1732 cm^{-1} indicated the presence of olefin, saturated and ester groups.

The ^{1}H NMR spectra (Appendix 3) showed multiplet peaks at δ 5.49 and 5.43 integrated for one proton each indicated the presence of olefin protons. A triplet peak at δ 4.10 integrated for two protons clearly revealed CH_2 group attached to heteroatom and other CH_2 group. A multiplet peak at δ 2.90 integrated for two protons showed protons attached to carbons that bear heteroatom. Two multiplet peaks at δ 2.35 and 2.18 integrated each for one proton indicated diastereotopic protons of CH_2 group. A triplet peak at δ 2.27 integrated for two protons revealed CH_2 group attached to carbonyl carbon and other CH_2 group. A multiplet peak at δ 2.05 integrated for two protons showed a CH_2 group.

A multiplet peak at δ 1.68 integrated for one proton indicated aliphatic CH proton. A quintet peak at δ 1.61 integrated for two protons showed CH_2 group attached to other two CH_2 groups. In addition, two CH_2 groups at δ 1.52, eight CH_2 groups at δ 1.35-1.26, and three CH_3 groups at δ 0.93-0.89 were observed.

The ^{13}C NMR spectra (Appendix 4) of **VG-3** displayed signals characteristics of an ester group (δ 173.57), a disubstituted double bond (δ 123.94, 132.33), a carbon next to heteroatom (O) which is CH_2 δ 62.69, and two CH carbon atoms attached to heteroatom (O) at δ 56.93 and 56.30. It also revealed the presence of aliphatic CH, CH_2 and CH_3 groups at δ 37.36-13.86.

The DEPT experiment (Appendix 4) showed 24 carbon resonances corresponding to one quaternary, five methine, sixteen methylene and three methyl carbon atoms (Table 3).

Table 3. ^{1}H-NMR and ^{13}C-NMR data of **VG-3** (Compound **31**)

No. of C/H	δ ^{1}H (ppm)	δ ^{13}C (ppm)	Remark
1	------	173.57	quaternary
2	2.27, t J= 6.8 Hz	34.22	CH_2
3	1.61, quintet	24.88	CH_2
4	1.35-1.26, complex	28.99	CH_2
5	1.35-1.26, complex	29.02	CH_2
6	1.35-1.26, complex	29.05	CH_2
7	1.35-1.26, complex	29.40	CH_2
8	2.05, m	25.98	CH_2
9	5.49, m	132.33	CH
10	5.43, m	123.94	CH
11	2.18, m 2.35, m	26.22	CH_2
12	2.90, m	56.93	CH
13	2.90, m	56.30	CH
14	1.52, m	27.69	CH_2
15	1.35-1.26, complex	27.31	CH_2
16	1.35-1.26, complex	31.66	CH_2
17	1.35-1.26, complex	22.49	CH_2
18	0.93-0.89, complex	13.86	CH_3
19	4.10, t J= 7.6 Hz	62.68	OCH_2
20	1.52, m	37.36	CH_2
21	1.68, m	34.10	CH
22	1.35-1.26, complex	26.19	CH_2
23	0.93-0.89, complex	16.29	CH_3
24	0.931-0.889, complex	22.35	CH_3

Table 4. Comparisons of selected ^{1}H and ^{13}C NMR spectra of **VG-3** and literature value of methyl cis-12, 13-epoxyoleate (vernolate).

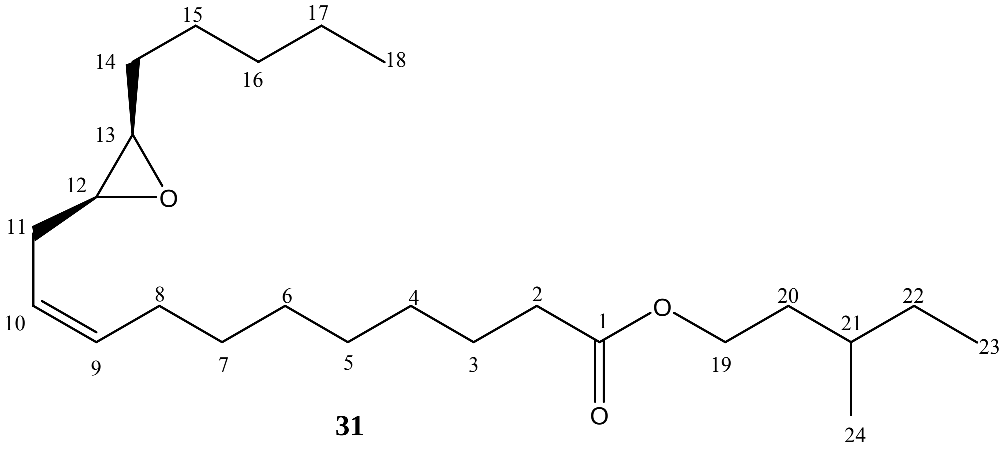
No. of H/C	δ ^{1}H (ppm) of VG-3	δ ^{1}H (ppm) of methyl cis-12,13-epoxyoleate(vernolate) [54]	δ ^{13}C (ppm) of VG-3	δ ^{13}C (ppm)of methyl cis-12,13-epoxyoleate(vernolate)[55]
1			173.54	173.81
2			34.17	33.75
3			24.82	24.63
4			28.98	28.80
5			29.02	28.80
6			29.07	28.80
7			29.41	29.20
8			25.99	25.99
9	5.49, m	5.43, m	132.24	132.19
10	5.41, m	5.33, m	123.94	123.78
11	2.18, m 2.35,m	2.10, m 2.27, m	26.22	25.99
12	2.90, m	2.82, m	56.94	56.79
13	2.90, m	2.82, m	56.30	56.16
14	1.52, m	1.44, m	27.69	27.49
15			27.31	27.11
16			31.66	31.46
17			22.49	22.27
18			13.86	13.63
19			62.68	-
20			37.36	-
21			34.10	-
22			26.19	-
23			16.29	-
24			22.351	-

Comparison of the ^{13}C-NMR and ^{1}H-NMR spectra (see Appendix 3 and 4) of **VG-3** with methyl cis-12, 13-epoxyoleate (vernolate) reported in the literature (Table 4) showed a good agreement. How ever, VG-3 has six additional carbon atoms instead of methyl groups in vernolate. There fore, VG-3 is most likely an ester of vernolic acid (derivative of vernolic acid).

In general the epoxide carbon atoms have chemical shifts of around 56.9 (cis) and 58.5 ppm (trans), but these may appear as two different signals through the long range influence of a carboxyl group or the omega methyl function [55]. In addition most epoxy carbons have signals for the protons attached to epoxidized carbons at δ 2.7 ppm for cis and δ 2.45ppm for trans [54].

Further more the current evidences based on the incorporation of oxygen atom to the double bond of linoleate to vernolate by peroxygenase enzyme (as reported by Bafor et al. 1993) that possess regio and enantio-selectivity forming exclusively the 12(S), 13(R)-enantiomer of vernolic acid is consistent with our prediction.

There fore the proposed structure for **VG-3** could be:



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Moreover, the above prediction is supported by 2D NMR spectral data as follows.

^{1}H-^{1}H Correlation Spectroscopy (COSY) (Appendix 5) revealed strong correlations among protons (Table 5).

Table 5. ^{1}H-^{1}H COSY of VG-3 (Compound **31**)

H on carbon No.	δ ^{1}H-NMR (ppm)	^{1}H-^{1}H COSY
2	2.27, t	H_2-H_3
3	1.66, quintet	H_3-H_2, H_3-H_4
4	1.35-1.26, complex	H_4-H_3

7	1.35-1.26 , complex	H_7-H_8
8	2.05, m	H_8-H_7, H_8-H_9
9	5.49, m	H_9-H_8, H_9-H_{10}
10	5.43, m	H_{10}-H_9, H_{10}- H_{11a}, H_{10}-H_{11b}
11	a) 2.18, m b) 2.35, m	H_{11a}-H_{11b}, H_{11a}-H_{10}, H_{11a}-H_{12} H_{11b}-H_{11a}, H_{11b}-H_{10}, H_{11b}-H_{12}
12	2.90, m	H_{12}-H_{11a}, H_{12}-H_{11b}, H_{12}-H_{13}
13	2.90, m	H_{13}-H_{12}, H_{13}-H_{14}
14	1.52, m	H_{14}-H_{15}
17	1.35.26 , complex	H_{17}-H_{18}
18	0.93-0.89, complex	H_{18}-H_{17}
19	4.09, t	H_{19}-H_{20}
20	1.52, m	H_{20}-H_{19}, H_{20}-H_{21}
21	1.68, m	H_{21}-H_{20}, H_{21}-H_{24}
22	1.35-1.26 , complex	H_{22}-H_{23}
24	0.93-0.89, complex	H_{24}-H_{21}

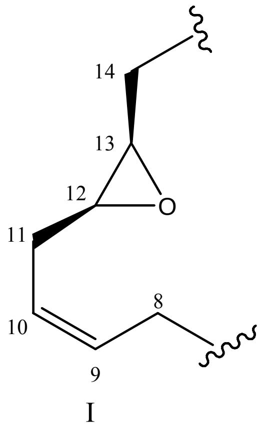
Hetronuclear **S**ingle **Q**uantum **C**orrelation (**HSQC**) experiment correlates the chemical shift of proton/s with the chemical shift of directly bonded carbon. The HSQC spectra of VG-3 (Appendix 6) showed a proton at δ 5.49 (m) attached to C-9 δ 132.33, a proton at δ 5.43 (m) connected with C-10 δ 123.94, two protons at δ 4.10 (t) attached to C-19 δ 62.68 and two protons at δ 2.90 (m) connected with C-12 and C-13 at δ 56.93 and 56.30 respectively. In addition, two protons at δ 2.35 (m) and 2.18 (m) attached to C-11 δ 26.22, hence they are diastereotopic protons. Two protons at δ 2.27 (t) attached to C-2 δ 34.22, two protons at δ 2.05 (m) connected with C-18 δ 25.98, one proton at δ 1.66 (m) connected with C-12 δ 34.10, two protons at δ 1.61 (quintet) attached to C-3 δ 24.88, four protons at δ 1.52attached to C-14 and C-20 at δ 27.69 and 37.36 respectively. Finally, sixteen methylene protons at δ 1.35-1.26 attached to carbons at δ 31.66-22.49 and nine methyl protons at δ 0.93-0.89 attached to carbons at δ 22.35-13.86.

Hetronuclear Multiple Bond Correlation (HMBC) experiment gives information about coupling of hydrogens and carbons that are two or three bonds away. The HMBC (see Appendix 7) of **VG-3** correlation between carbon and protons is presented in (Table 6).

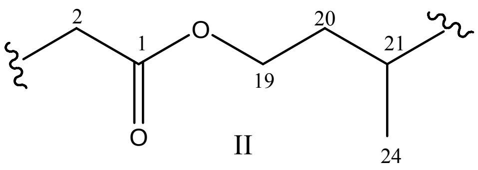
Table 6. HMBC Correlation of **VG-3** (Compound **31**)

C No.	δ ^{13}C(ppm)	HMBC (^{1}H $\longrightarrow$ ^{13}C)
1	173.54	H-2 (δ 2.24), H-3 (δ 1.61), H-19 (δ 4.10)
2	34.22	H-3 (δ 1.61), H-4 (δ 1.35- 1.26)
3	24.88	H-4 (δ 1.35-1.26)
4	28.99	H-2 (δ 2.27), H-3 (δ 1.61)
5	29.02	H-3 (δ1.61), H-6 and H-7 (δ 1.35-1.26), H-8 (δ 2.05)
6	29.05	H-7 (δ 1.35-1.26), H-8 (δ 2.05), H-9 (δ 5.49)
7	29.40	H-6 (δ 1.350-1.26), H-8 (δ 2.05), H-9 (δ 5.49)
8	25.98	H-7 (δ 1.350-1.26), H-9 (δ 5.49)
9	132.33	H-7 (δ 1.35-1.26), H-8 (δ 2.05), H-10 (δ 5.43)
10	123.94	H-8 (δ 2.05), H-9 (δ 5.49), H-11 (δ 2.18 and 2.35)
11	26.22	H-9 (δ 5.49), H-10 (δ 5.43), H-12 and H-13 (δ 2.90), H-14(δ 1.52)
12	56.93	H-9 (δ 5.49), H-10 (δ 5.42), H-11 (δ 2.18 and 2.35), H-13 (δ 2.90), H-14(δ 1.52)
13	56.30	H-10 (δ 5.43), H-11 (δ 2.18 and 2.35), H-12 (δ 2.90), H-14(δ 1.52)
14	27.69	H-11 (δ 2.18 and 2.35), H-12 and H-13 (δ 2.90, H-15 (δ 1.35-1.26)
15	27.31	H-13 (δ 2.90), H-14(δ 1.52)
16	31.66	H-14(δ 1.52), H-15 and H-17 (δ 1.350-1.26), H-18 (δ 0.93-0.89)
17	22.49	H-18 (δ 0.93-0.89), H-16 (δ 1.35-1.26)
19	62.68	H-20 (δ 1.52), H-21 (δ 1.68)
20	37.36	H-21 (δ 1.68), H-22 (δ 1.35-1.26), H-24 (δ 0.93-0.89)
21	34.10	H-20 (δ 1.52), H-22 (δ 1.35-1.26), H-23 (δ 0.93-0.89), H-24 (δ 0.93-0.89)
22	26.19	H-24 (δ 0.93-0.89), H-21 (δ 1.66)
23	16.29	H-22 (δ 1.35-1.26), H-24 (δ 0.93-0.89)

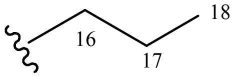
Based on the COSY, HSQC and HMBC spectra, the partial structures (I), (II) and (III) are predicted.



I



II



III

Connecting these fragments (partial structures) the following structure could be evident.

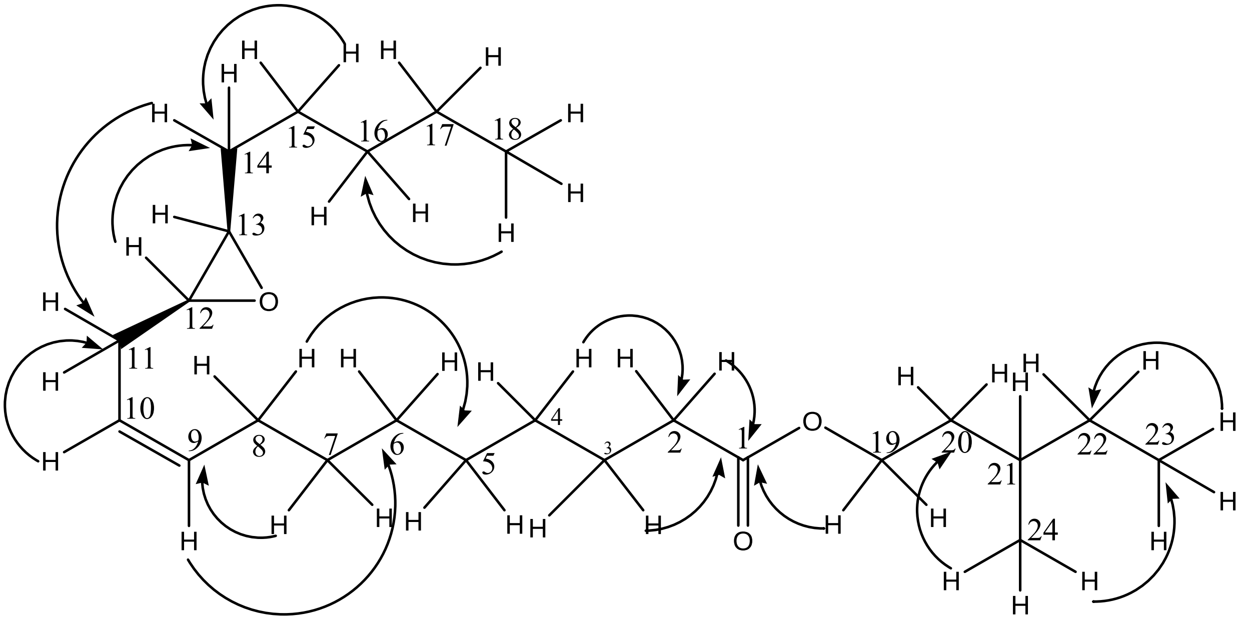


Fig. 10. The Selected HMBC Correlation of VG-3

3.3.4. Partial Characterization of VG-4A (Compound 32)

The spectral data for **VG-4A** showed the presence of functional groups which are similar to that of **VG-3**. Hence, the skeleton of **VG-3** and **VG-4A** is similar to methyl vernolate. But they differ in their over all structures. Keeping vernolate moiety as it was, partial characterization of this compound could be as follow.

The IR spectrum (Appendix 8) of **VG-4A** has a band at 3454 cm^{-}, which indicated the presence of OH group of an alcohol. The ^{1}H NMR spectra (Appendix 9) of **VG-4A** showed doublet peak at 4.14 integrated for two protons indicated CH_2 group attached to heteroatom (O) and other CH group. A multiplet peak at δ 3.9 integrated for one proton showed a CH group attached to heteroatom. Doublet peaks at δ 3.78 and 3.66 integrated for one proton each showed distereotopic protons of CH_2 group that attached to heteroatom.

Both the ^{13}C NMR and DEPT experiments (Appendix 10) of VG-4A showed the presence of CH_2 group at δ 65.10 (C-19), one CH group at δ 70.24 (C-20) and one CH_2 group at δ 63.41 (C-21) which are attached to hetero atoms (O).

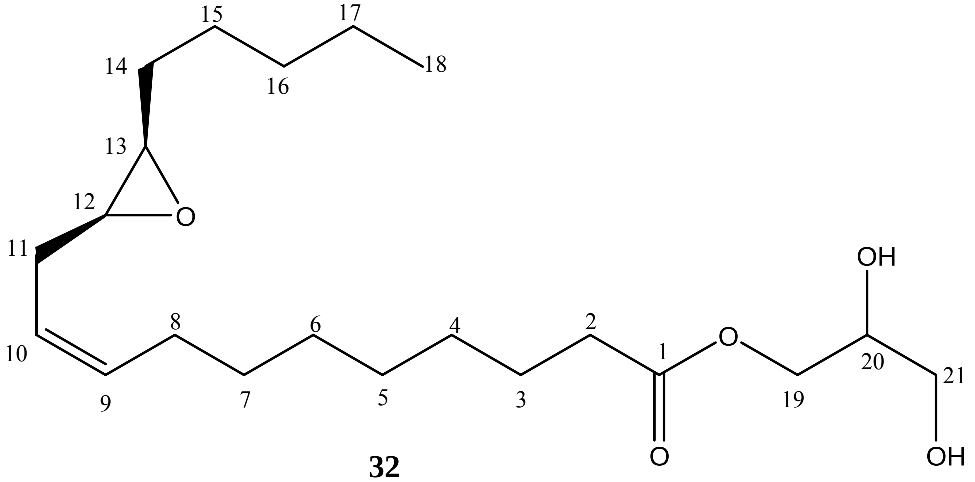
Table 7. Comparison of ^{13}C NMR of **VG-3** and **VG-4A.**

No. of C	δ ^{13}C NMR(ppm) **VG-3**	δ^{13}CNMR(ppm)**VG-4A**
1	173.54	174.27
2	34.17	34.14
3	24.82	24.82
4	28.98	29.06
5	29.02	29.12
6	29.07	29.47
7	29.41	29.69
8	25.99	26.20
9	132.24	132.60
10	123.94	123.89
11	26.22	26.24
12	56.94	57.41
13	56.30	56.74
14	27.69	27.71
15	27.31	27.39
16	31.66	31.73
17	22.49	22.57
18	13.86	13.98
19	62.68	65.10

20	37.36*	70.24*
21	34.10*	63.41*
22	26.19*	-----
23	16.29*	------
24	22.35*	------

- indicates the difference between **VG-3** and **VG-4A**

Therefore the probable structure of **VG-4A** could be:



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The 2D spectra experiment was not carried out for this sample due to its similarity with that of **VG-3** as well as its small amount and extra information was not obtained to be sure about the above structure.

4. EXPERIMENTAL

4.1. Plant materials

Ethiopia Agricultural Research Instititute (EARI), Essential Oils Research Center (EORC), provided seed materials, which were collected from different regions of the country.

V_1- VAT-3 plot 2: collected from southern Ethiopia around Dilla at altitude of 1550 m.

V_2- VAT-3 plot 4: collected from southern Ethiopia around Dilla at altitude of 1550 m

V_3- VAT-2 plot 3: collected from Meki and Bulbula regions around Zeway at altitude of 1600 m.

V_4-VAT-3 plot 5: collected from southern Ethiopia around Dilla at altitude of 1550 m.

V_5-VAT-2 plot 11: collected from Meki and Bulbula regions around Zeway at altitude of 1600 m.

All voucher specimens are deposited at National Herbarium, Department of Biology, Addis Ababa University.

4.2. General

UV spectrum was measured with GENESY'S Spectrometer (200-400 nm) in $CHCl_3$ at room temperature. IR spectrum was measured with Perkin Elmer BX Infrared spectrometer in the range 4000-400 cm^{-1}

^1H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer with TMS as internal standard.

Optical rotation was measured with P20 polarimeter at room temperature. Refractive index was recorded by Abbe's refractometer and Specific gravity was determined by using Pycnometer; all measurements were conducted at 20^0C.

Silical gel with fluorescent indicator 254 nm on aluminum cards with layer thickness 0.2 mm was used for TLC and Silicalgel 60 (Merck), particle size of 0.063-0.200 mm was used for column chromatography.

4.3. Extraction of vernonia oil.

Dried and powdered seeds of five accessions of *V. galamensis* were extracted with n-hexane for three hour using Soxtect1040 and Soxhelet extraction systems. The oil yield (percentage of the oil) in each accession was determined and the crude oil was subjected to refining process. Physicochemical characteristics of the refined oil were then measured.

4.4. Refining of Vernonia oil

In addition to triglycerides, the crude oil contains variable amounts of objectionable substances, which must be removed to produce a finished oil of good quality. Some of these impurities are of the non-glyceride type such as the free fatty acids (FFA), which may be built up due to enzymatic processes (lipase) resulting from damage to the seed. While others are of the non oil kind such as the phsphatide or mucilage volatile including moisture and solvent, pigment or coloring materials primary and secondary oxidation products, waxes and saponifiable and odoriferous materials [12, 13, 56]. Hence refining steps proceeded were:

4.4.1. Bleaching

Crude vernonia oil was refined with activated charcoal. 5% to 8% by weight of activated charcoal was mixed with the oil and heated at a temperature of 60^0C by a continuous stirring for one hour. The decolorized oil was isolated by hot filtration.

4.4.2. Degumming

Almost all seed oils contain impurities in the colloidal state or dissolved in them. These substances must be removed from the oil. This purification process is known as deguming and is usually carried out immediately before neutralization or concurrently with it. Crude vernonia oil was degummed by stirring with 2.5-5 g by weight of distilled water at 60^0C for one hour followed by centrifugation at 2,800 rpm. for 2-3 hr. Gum and oil were separated and the oil was dried at 60^0C on a rotary evaporator.

4.4.3. Neutralization

Organic acids, which are always dissolved in the oil, are removed by saponification with sodium hydroxide solution. Separations occur easily because the resulting soaps are practically insoluble in the neutral oil under standard operation conditions. In the neutralization processes sodium hydroxide solution of different strengths are used according to the FFA content and quality of the oils. For FFA contents below one percent, more dilute solution (8% to 12%) are employed, where as for higher FFA contents strength of about 20% are required.

The degumed vernonia oil was mixed with sodium hydroxide solution and then heated to 40^0C followed by stirring for 30 min. From the mixer the oil-soap stock suspension passes through the centrifugal separator, which separates the soap stock from the neutral oil. Oil was dried for one hour on a rotary evaporator at 60^0C.

4.4.4. Re-refining of the neutral oil

The neutral oil obtained from the neutralization contains minute quantities of FFAs and other impurities. Such impurities were removed by treatment with a dilute solution of sodium hydroxide and sodium carbonate. The purpose of re-refining is to remove the last trace of fatty acids, phosphatides etc. from the neutralized oils.

4.4.5. Washing of the oil

To obtain soap free oil after the degumming, neutralization and re-refining steps, a vigorous washing by hot water were carried out. This is necessary because the soaps are always partially soluble in the neutral oil.

4.5. Extraction and Isolation.

4.5.1. Extraction of plant material

400 g of dried and grounded seeds of *V. galamensis* was soaked in 0.9 L of dichloromethane for 36 hour and the extract was freed of solvent to give 90 g of thick oil. Further extraction of the marc with 0.85 L of methanol for 48 hour was done and the extract was concentrated to yield 43 g of residue (Figure 11). Application of TLC and column chromatography resulted in isolation of **VG-3** and **VG-4A** as detailed below.

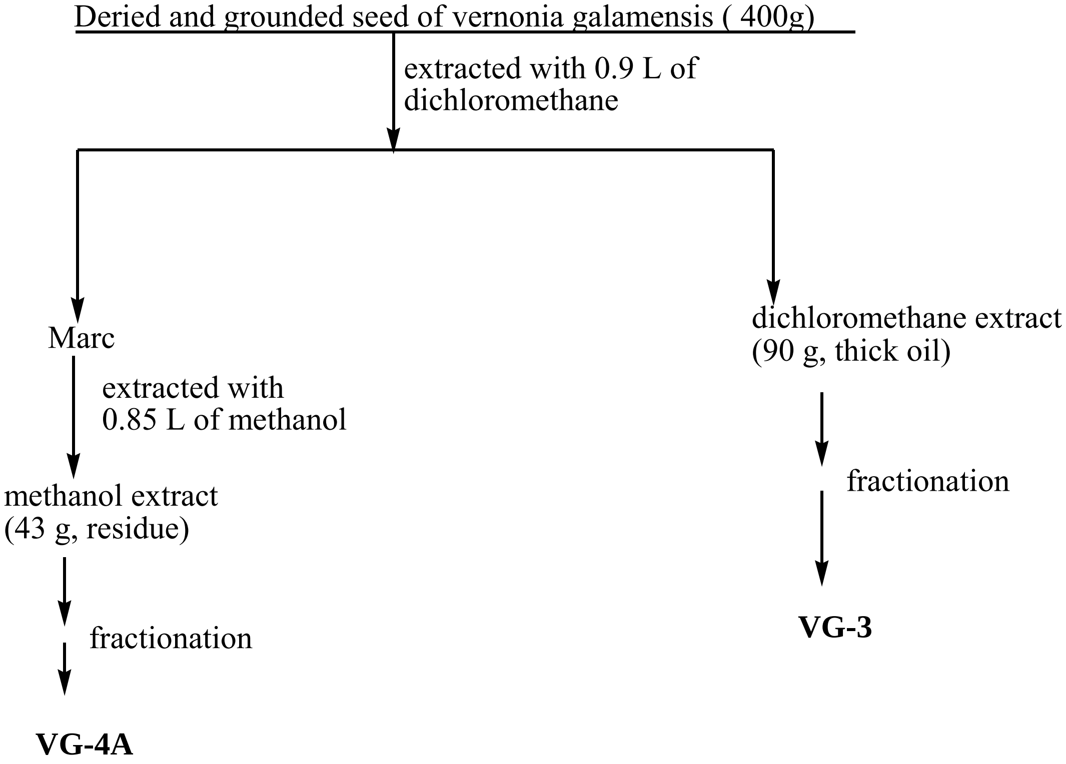

Figure 11. General out line scheme of solvent extraction for *V. galamensis* seed

4.5.2. Fractionation of dichloromethane extract

30 g of the thick oil extract of dichloromethane was subjected to column chromatography in which 130 g of Silica gel was packed with n-hexane. Successive elution with increasing polarity resulted in 12 fractions (Table 8).

DCM-1 was subjected to column chromatography and elution with increasing polarity in the same solvent continued to yield 5 fractions. The second fraction [which was eluted with n-hexane-dichloromethane (98:2)] was passed through column chromatography and 12 fractions were collected. Fractions 3’-5’, which were eluted with n-hexane-dichlorometane (98:2) were then combined on the basis of TLC analysis (Figure 12). TLC application of this combined fraction gave single spot and was sent to spectra. The compound isolated was coded as **VG-3**. TLC analysis of DCM-2 showed mixtures of compounds. Hence, further analysis was not carried out on this fraction as well as other fractions of dichloromethane extract

Table 8. Solvent systems used in column chromatography for fractionation of dichloromethane extract

No.of fractions	Solvent systems	Volume (ml)
1	n-hexane (100%)	100
2	n-hexane-dichloromethane (98:2)	50
3	n-hexane-dichloromethane (95:5)	50
4	n-hexane-dichloromethane (85:5)	50
5	n-hexane-dichloromethane (70:30)	100
6	n-hexane-dichloromethane (1:1)	100
7	Dichloromethane (100%)	100
8	Dichloromethane-methanol (90:10)	50
9	Dichloromethane-methanol (80:20)	50
10	Dichloromethane-methanol (60:40)	75
11	Dichloromethane-methanol (1:1)	50
12	Methanol (100%)	100

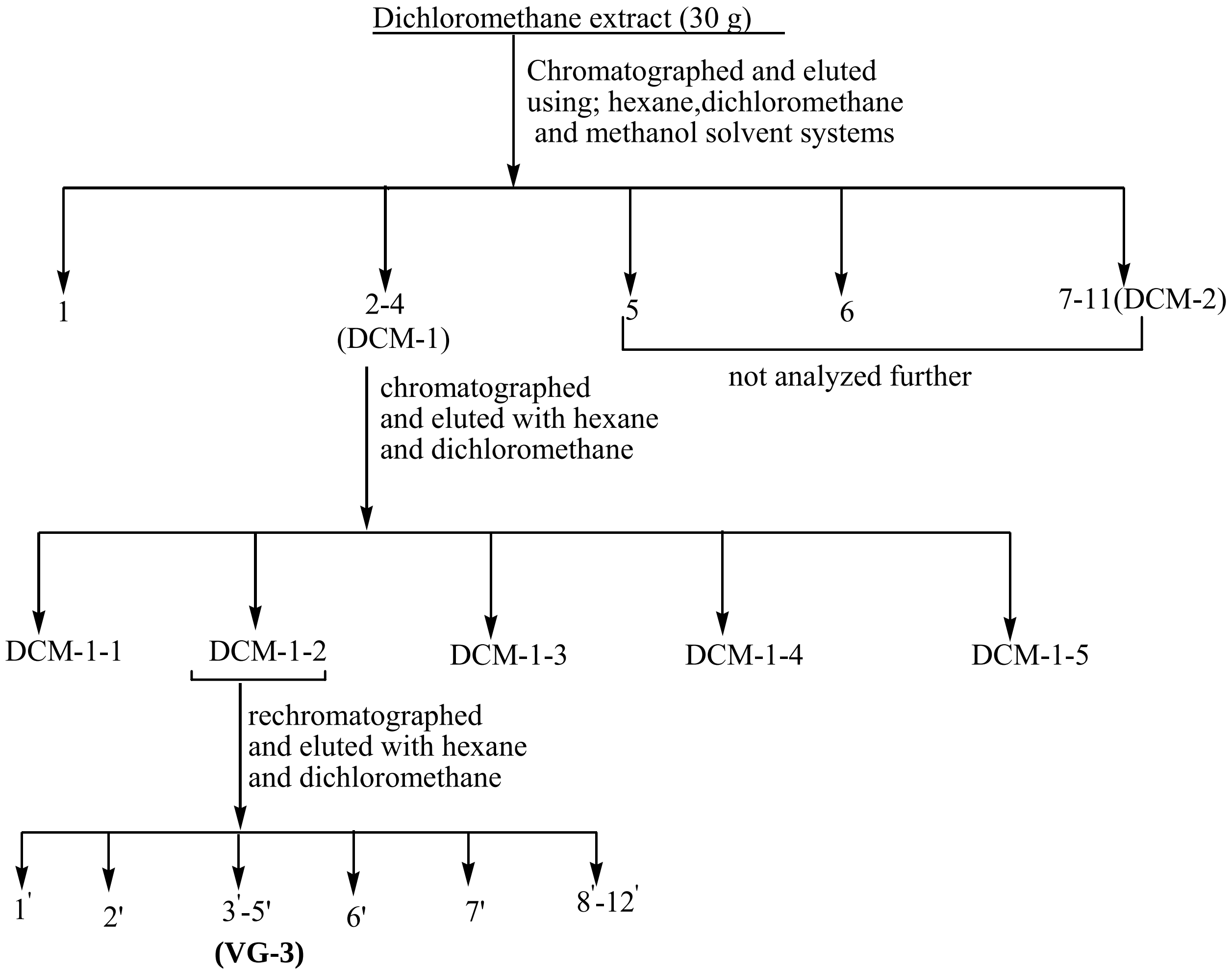

Fig 12. Fractionation scheme employed for dichloromethane extract of *V. galamensis* seed

4.5.3. Fractionation of methanol extract

25 g of methanol extract was subjected to column chromatography and successive elution resulted in 16 fractions (Table 9). Fractions 4-5, 8-10, and 11-15 were combined according to TLC application. Fraction 2 was chromatographed using chloroform-ethyl acetate solvent systems and 11 fractions were collected by increasing polarity. Fraction 6, a color less crystal of 200 mg [which was obtained from chloroform-ethyl acetate (80:20)] gave two spots on TLC analysis and then applied on to column chromatography. Elution with increasing polarity of chloroform-ethyl acetate solvent systems gave 10 fractions. This successive elution resulted in isolation of VG-6-2 and VG-6-1 (with chloroform-ethyl acetate (90:10) and (1:1) respectively). However their spectral data was not interpretable and their structures were not reported in this project.

Table 9. Solvent systems used in column chromatography for fractionation of methanol extract

No. of fractions	Solvent systems	Volume (ml)
1	Chloroform-ethyl acetate (1:1)	100
2	Chloroform-ethyl acetate (40:60)	100
3	Chloroform-ethyl acetate (30:70)	25
4	Chloroform-ethyl acetate (20:80)	75
5	Chloroform-ethyl acetate (10:90)	100
6	Ethy acetate (100%)	100
7	Ethyl acetate-Methanol (10:90)	100
8	Ethyl acetate-Methanol (80:20)	100
9	Ethyl acetate-Methanol (70:30)	100
10	Ethyl acetate-Methanol (60:40)	100
11	Ethyl acetate-Methanol (1:1)	100
12	Ethyl acetate-Methanol (40:60)	100
13	Ethyl acetate-Methanol (30 :70)	100
14	Ethyl acetate-Methanol (20:80)	100
15	Ethyl acetate-Methanol (10:90)	50
16	Methanol (100%)	50

The combined fractions of 4-5 were chromatographed using chloroform-ethyl acetate solvent system and resulted in 5 fractions. The second fraction [which was obtained by elution with chloroform-ethyl acetate (20:80)] gave a pure compound and coded as **VG-4A** (figure 13).

TLC analysis of fractions 8-10 as well as 11-16 showed tailed single spot in chloroform-methanol or chloroform-ethyl acetate solvent systems. However three spots were observed on TLC plate by using butanol-water-acetic acid (3:1:1) solvent system and methanolic KOH spray gave a yellow spot indicating the presence of flavonoids. However, pure compounds were not isolated and characterized from this fraction of plant material due to time constraint.

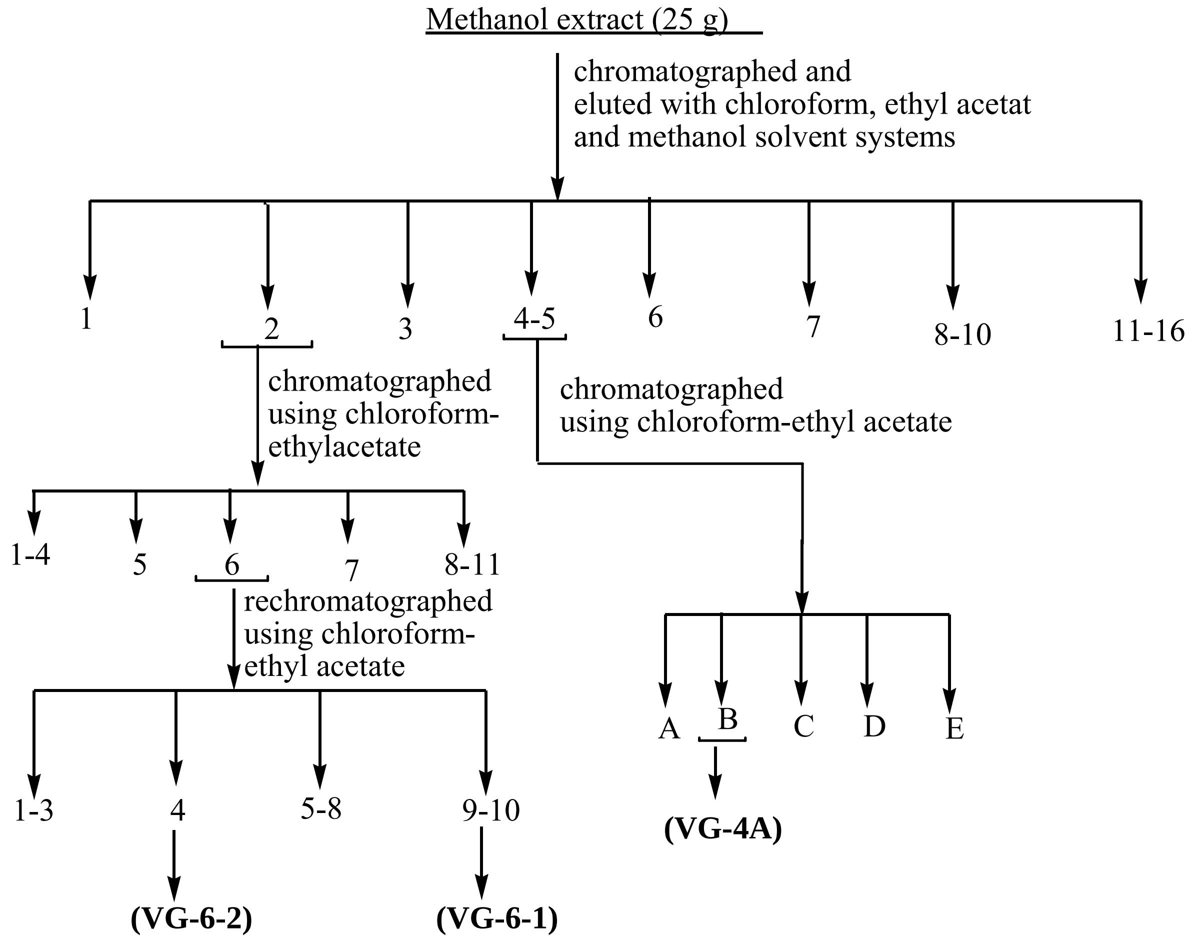

Fig. 13. Fractionation scheme employed for methanol extract of *V. galamensis* seed

4.6. Physico chemical data of the compounds isolated

VG-3 (compound **31**) with $R_f = 0.30$, was isolated as colorless oil from dichloromethane extract of the seed material.

IR ν_{max}, cm^{-1}: 3010 (CH=CH), 2928 (aliphatic CH_3, CH_2, CH), 1732 (an ester): see Appendix 2.

^1H NMR (400 MHz, $CDCl_3$): δ 5.49 (1H, m, H-9), δ 5.43 (1H, m, H-10), δ 4.10(2H, t, H-19), δ 2.90 (2H, m, H-12 and H-13), δ 2.35 (1H, m, H-11), δ 2.27 (1H, t, H-2), δ 2.18 (1H, m, H-11), δ 2.05 (2H, m, H-8), δ 1.68 (1H, m, H-21), δ 1.61 (2H, quintet, H-3), δ 1.52 (4H, m, H-14 and H-20), δ 1.35-1.26 (16H, complex, H-4 to H-7, H-15 to H-17 and H-22), δ 0.93-0.89 (9H, complex, H-18, H-23 and H-24): see Appendix 3.

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.54 (C-1, an ester quaternary carbon), δ 132.33 (C-9, an olefinic carbon), δ 123.94 (C-10, an olefinic carbon), δ 62.68 (C-19, $-OCH_2-$), δ 56.93 and 56.30 (C-12 and C-13, an epoxy carbons), δ 37.36 (C-20, aliphatic CH_2), δ 34.22 (C-2, aliphatic CH_2), δ 34.10 (C-21, aliphatic CH), δ31.66 to 22.49 12C, aliphatic methylenes), δ 22.35 to 13.86 (3C, aliphatic CH_3 groups): see Appendix 4.

VG-4A (compound **32**), with Rf=0.28 was isolated as yellow gummy from methanol extract of the seed material.

IR ν_{max}, cm^{-}: 3454 (OH), 2925 (aliphatic CH, CH_2, CH_3), 1740 (ester).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.14 (2H, d, H-19), δ 3.92 (1H, m, H-20), δ 3.78 (1H, d, H-21), δ 3.66 (1H, d, H-21).

^{13}C NMR (100 MHz, $CDCl_3$): δ 65.10 (C-19, CH_2 group attached to (O)), δ 70.24 (C-20, CH group attached to (O)), δ 63.41 (C-21, CH_2 group attached to (O)).

The remaining δ values for ^{1}H NMR and ^{13}C NMR spectra are more or less similar to that of **VG-3**.

5. Conclusions and Recommendations

Vernonia galamensis is a potential and new oil seed crop, which is essentially noncompetitive with the existing crops and possibly provides a new and unique product for industrial use particularly for marginal rainfall areas. The presence of epoxy group, the low viscosity and polymerizing characteristics of this oil makes it especially valuable as a solvent in industrial coatings and paints. Some of the products that are being developed from vernonia oil are also degradable lubricants and lubricant additives, epoxy resins, plastic formulations, adhesives, insecticides and insect repellants and reactive monomers in polymer synthesis.

A survey of literatures on vernonia species showed that most of the compounds isolated were flavonoids and sesquiterpene lactones and one of our first attempts in this project was to isolate and characterize these compounds.

The pure compounds isolated from dichloromethane extract as well as from fractions 1-5 of methanol extract showed common functional groups such as: an olefin, an epoxy and an ester groups with insignificant differences. This shows the presence of industrially important functional groups in the seeds of vernonia galamensis of Ethiopa. Since they are very crucial for the development of the country their cultivation should be intensified and diversified through out the country.

TLC analysis of fractions 8-10 as well as 11-16 of methanol extract gave tailed single spot in chloroform-methanol or ethyl acetate-methanol solvent systems. However three spots were observed on TLC plate by using butanol-water-acetic acid (3:1:1) solvent system and methanolic KOH spray gave a yellow spot probably indicating the presence of flavonoids. However, pure compounds were not isolated and characterized from this fraction of plant material for the following reasons. Since the boiling point of these solvents is high (greater or equal to 100^0C), it was not easily dried by rotary vapor at low temperature. Secondly, using rotary vapor at high temperature favors the formation of butyl acetate, which imparts impurity in NMR spectra. In the third case these fractions are not soluble in commonly available solvent ($CDCl_3$) or it requires more expensive solvents MeOD and DMSO. Finally, the spots are so close that (very close in polarity) separation by using column chromatography or preparative TLC was not successful. Further work to purify the compounds was not also performed due to time constraint.

Therefore researchers who are interested in this area can proceed using this solvent system under reduced pressure or other techniques such as HPLC or try other solvent systems.

6. References

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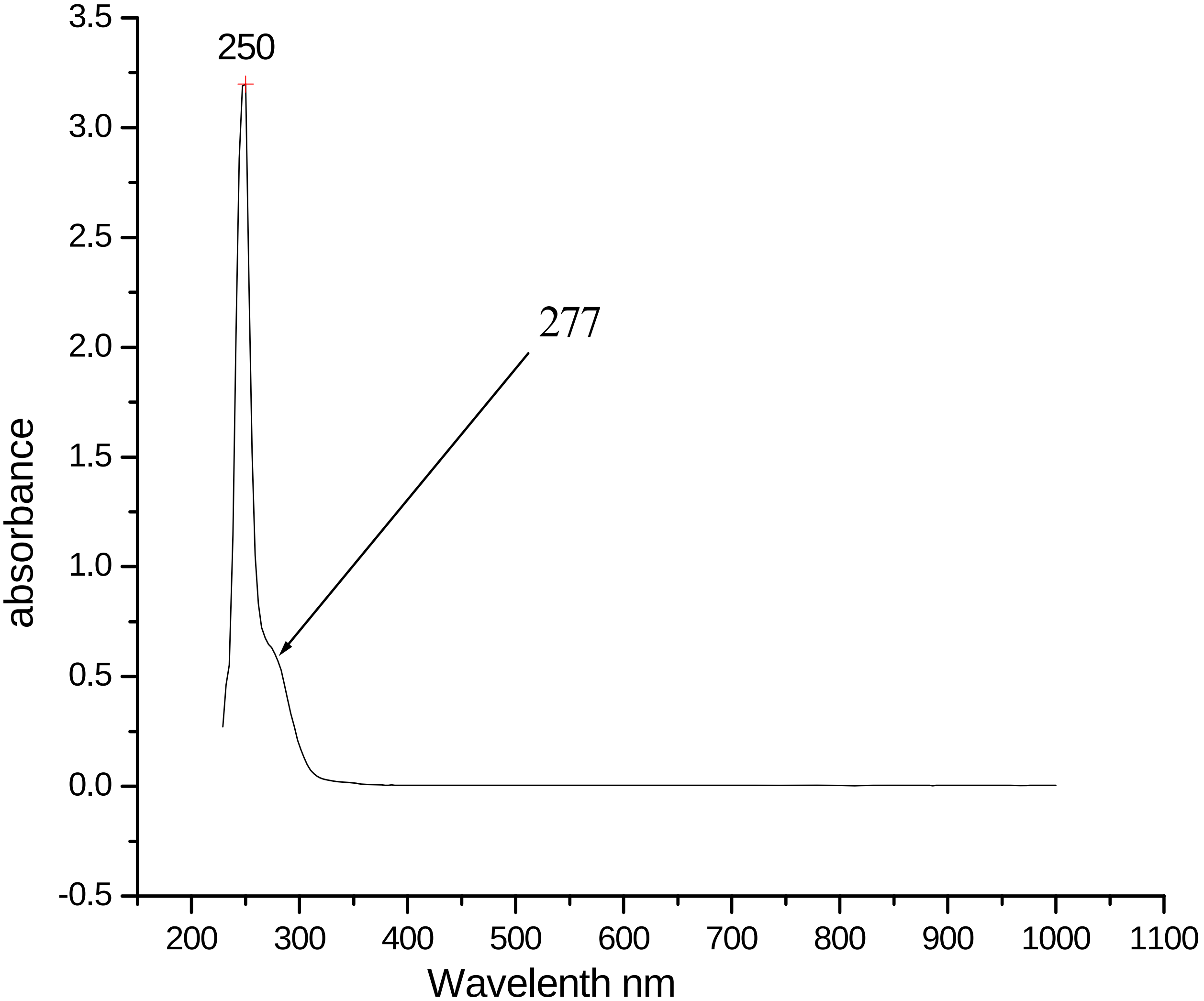
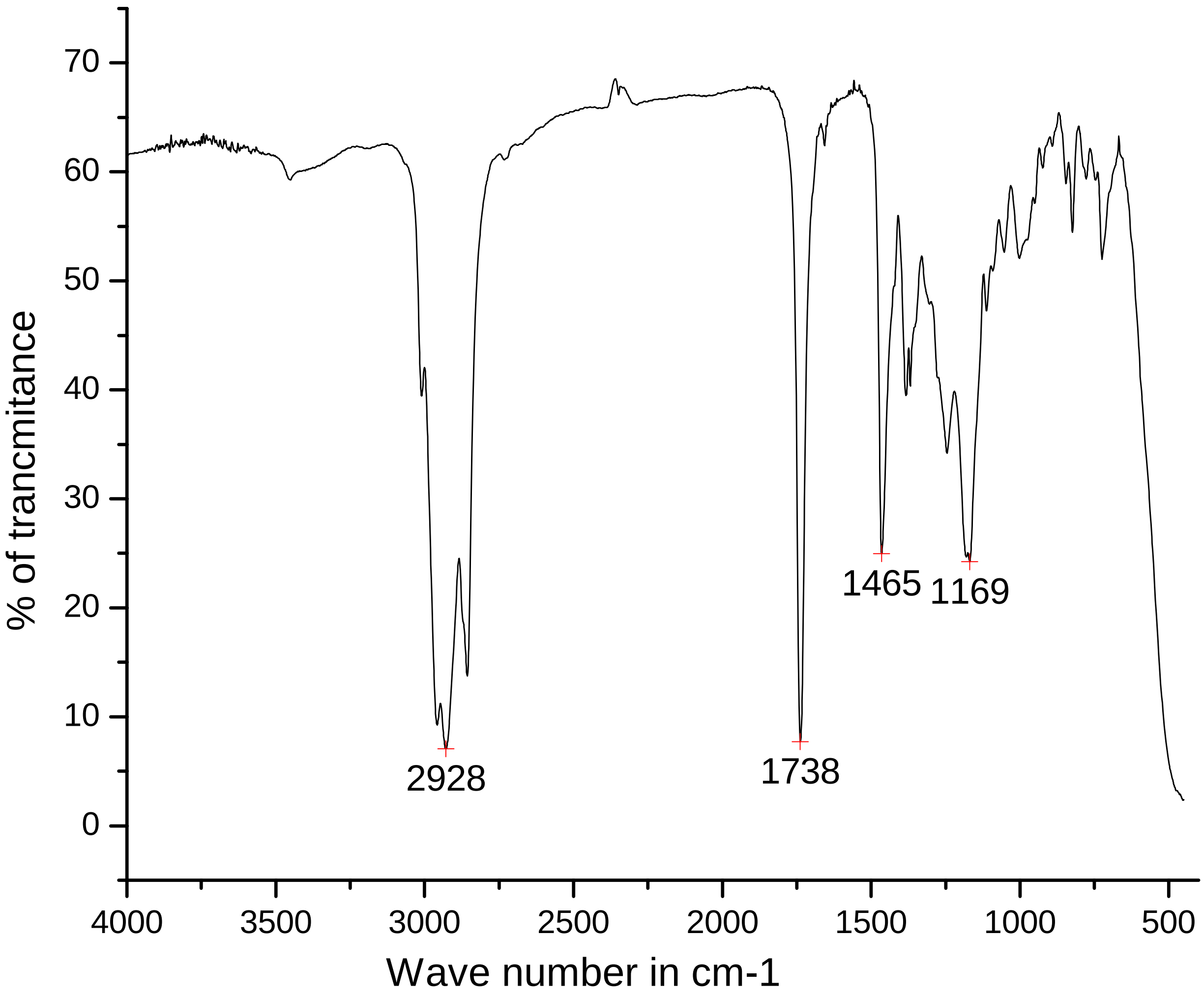
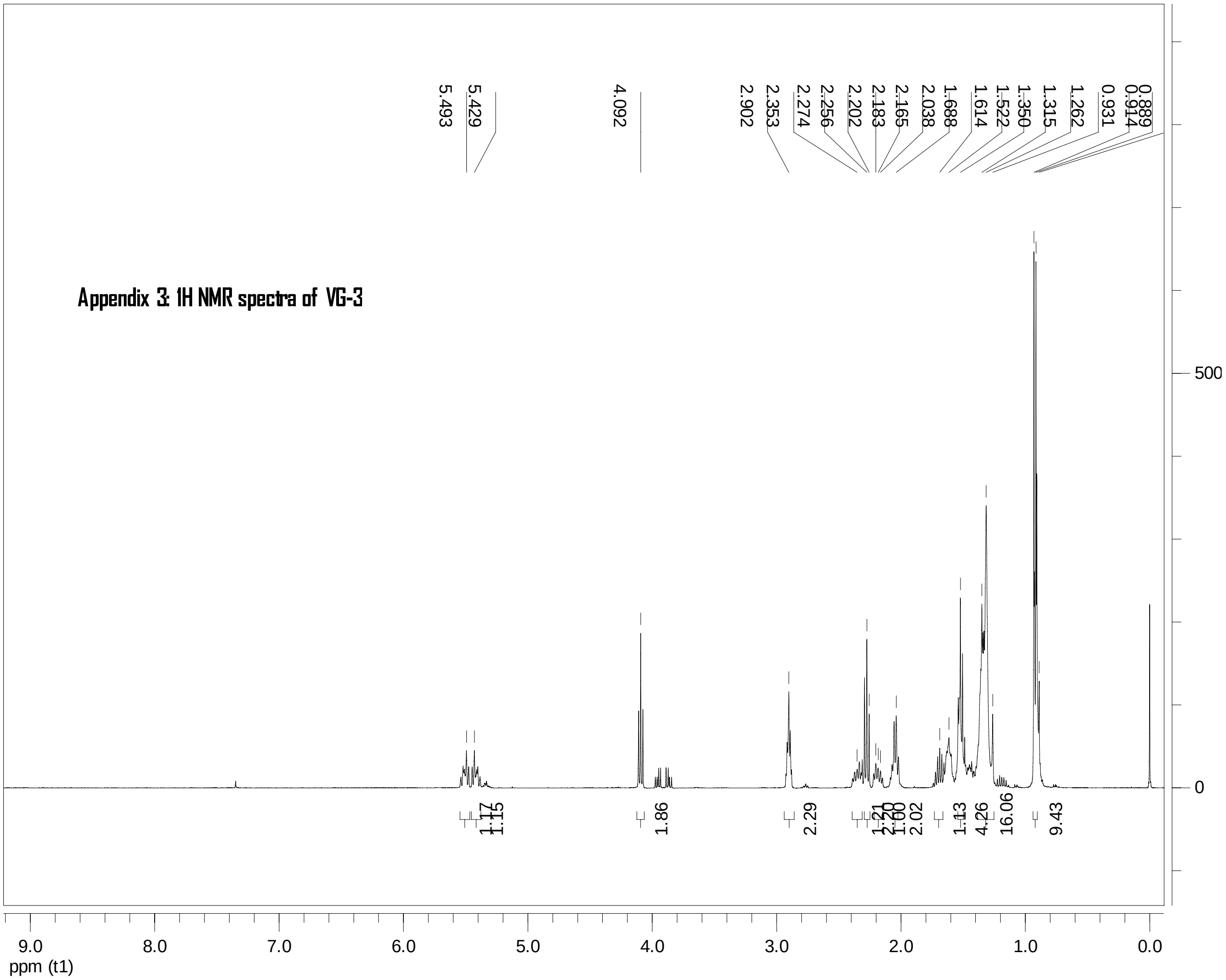
7. APPENDICES

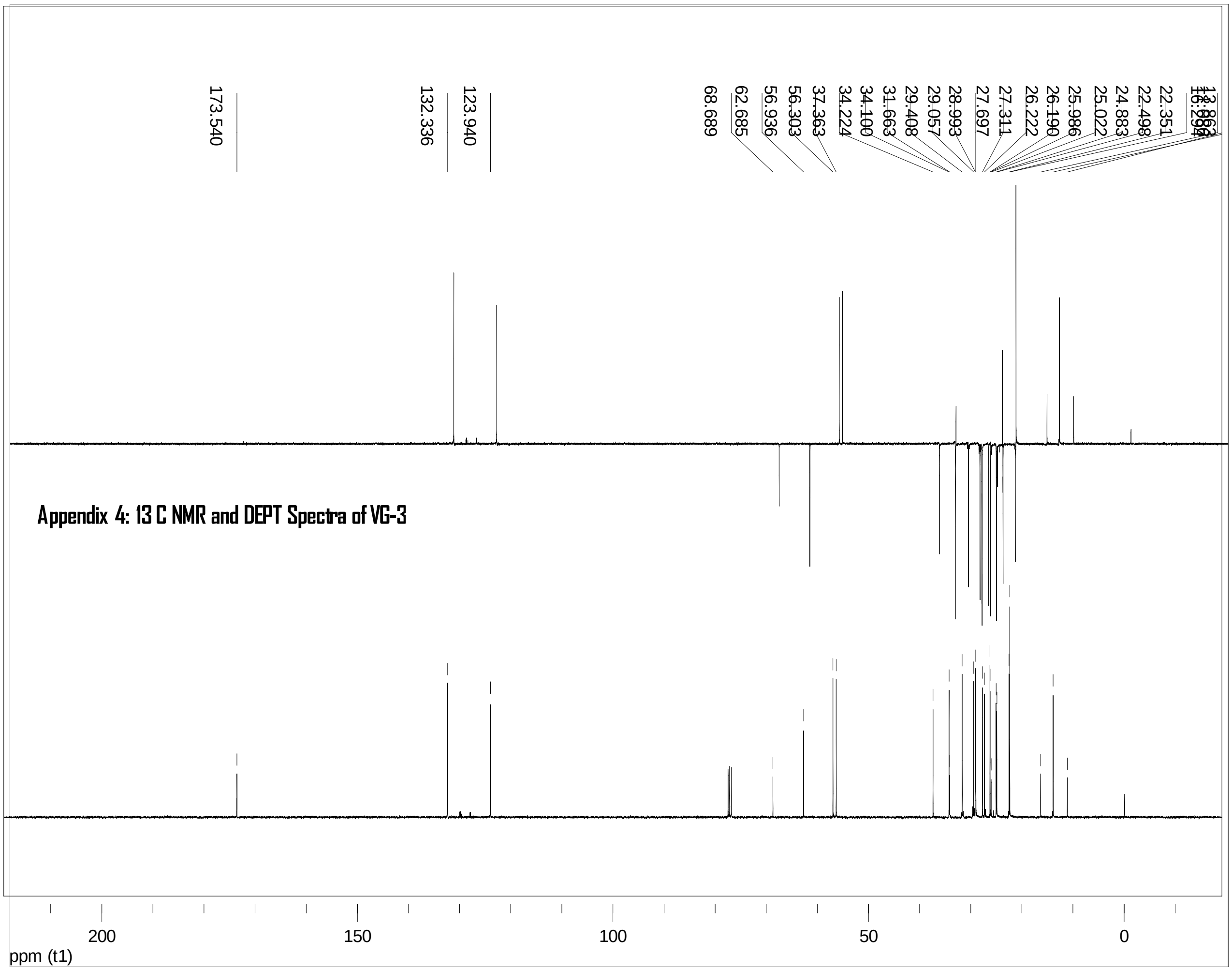


Appendix 1: UV-Vis spectrum of VG-3

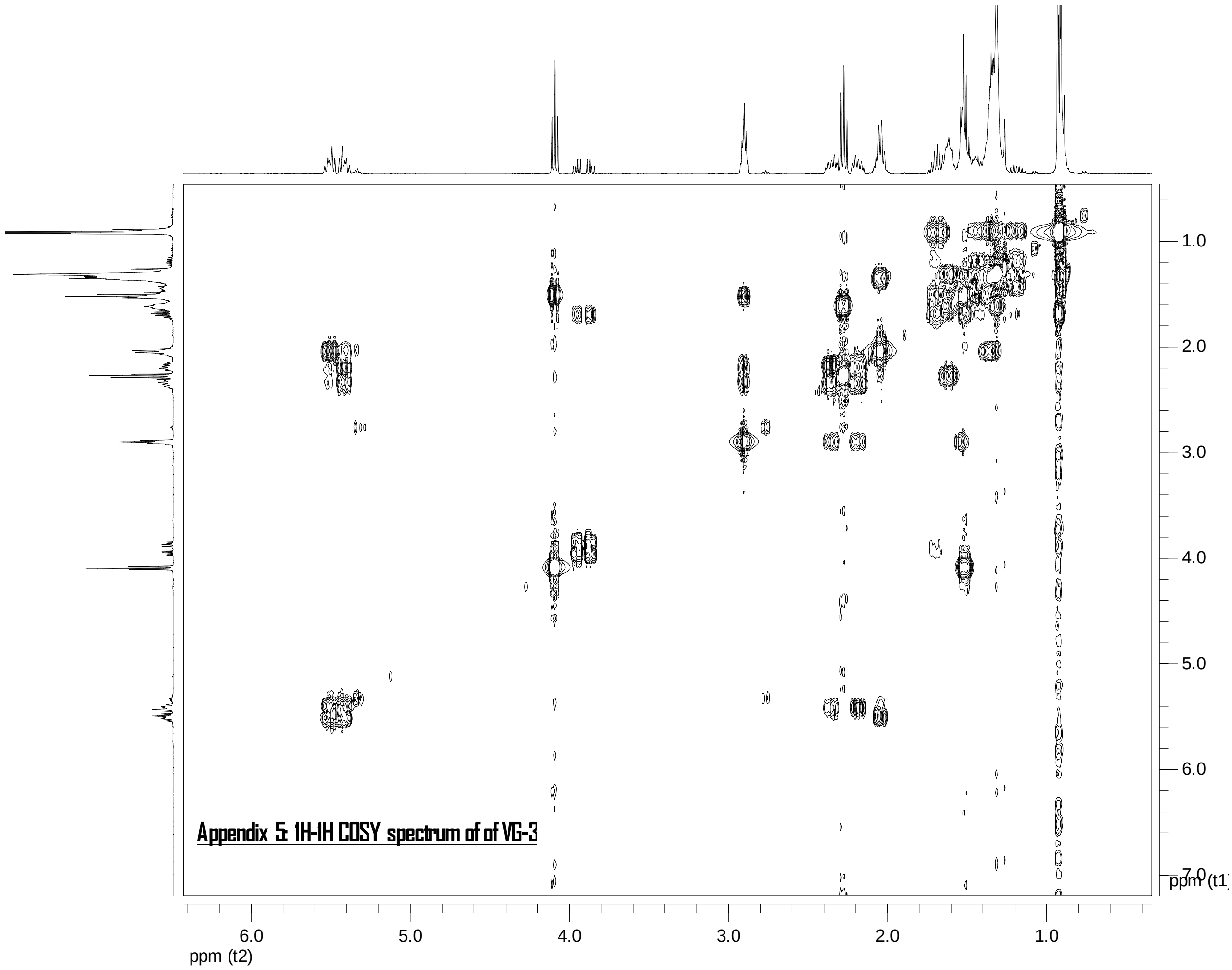


Appendix 2: IR spectrum of VG-3

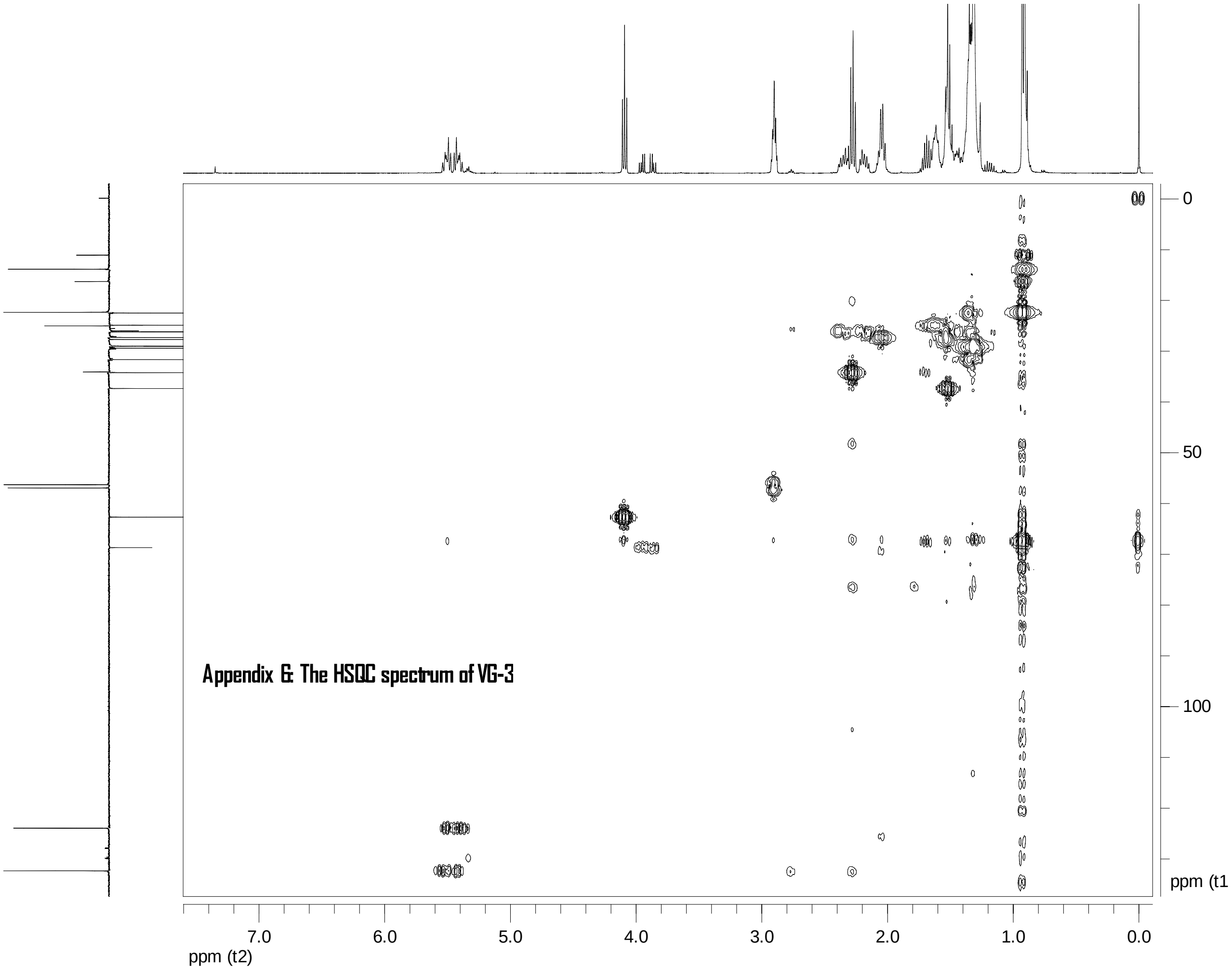

Appendix 3: 1H NMR spectra of VG-3



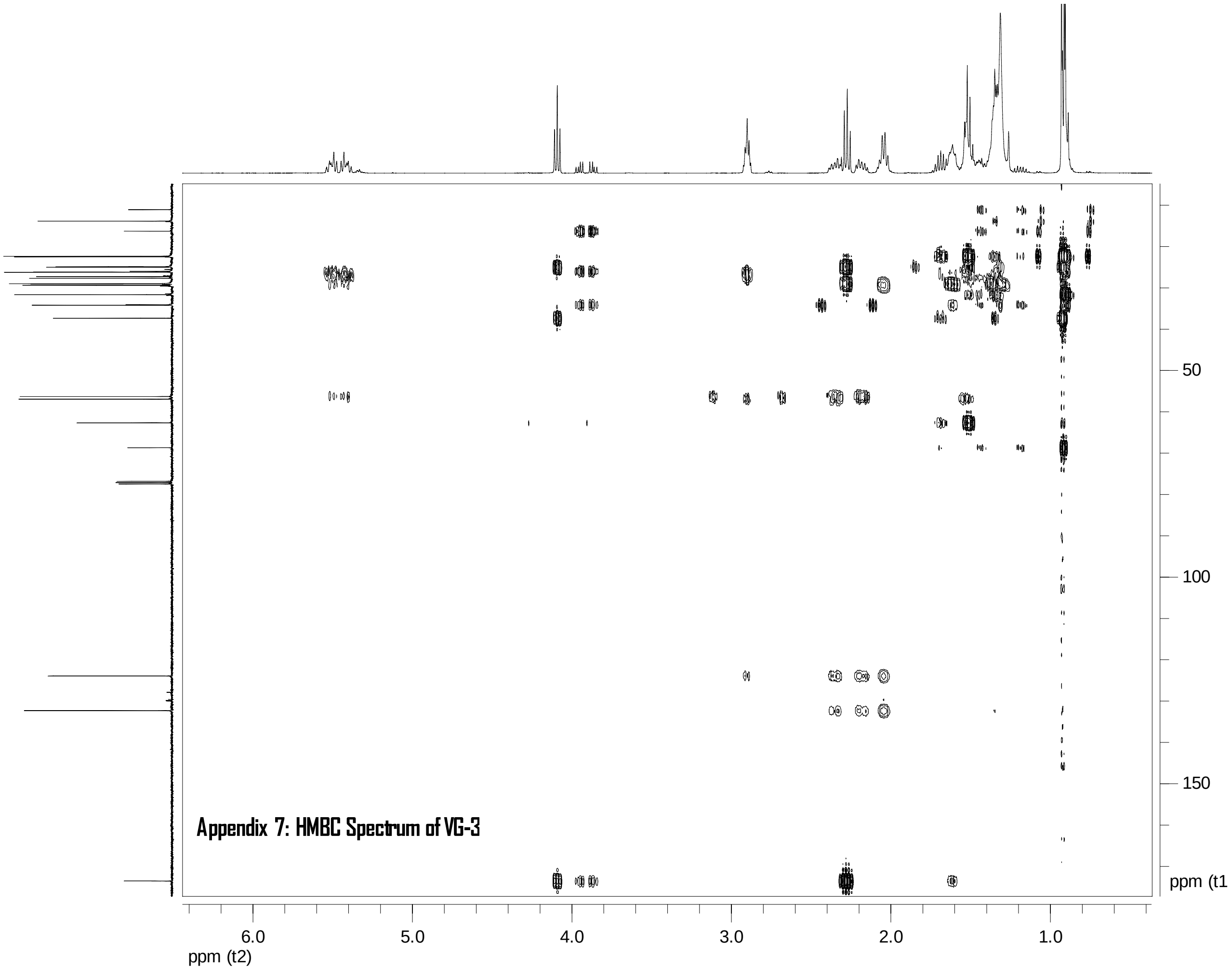
Appendix 4: 13 C NMR and DEPT Spectra of VG-3

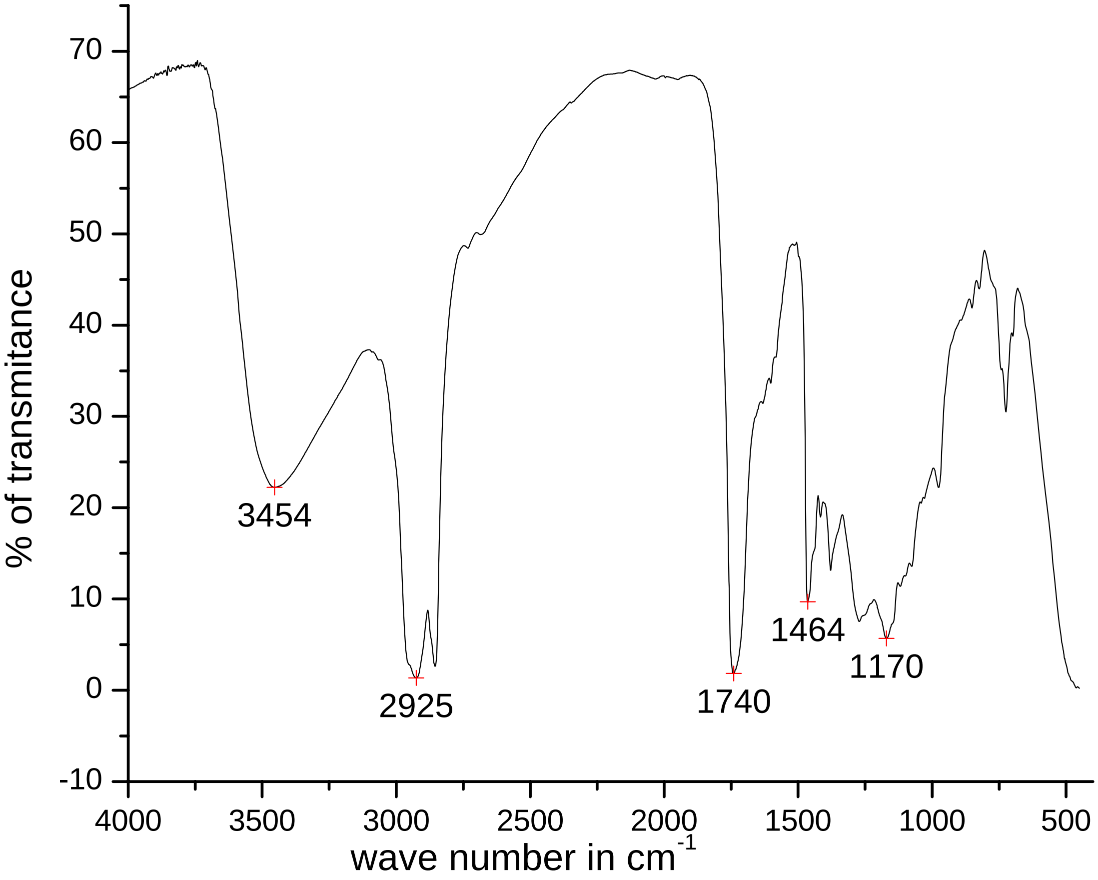


Appendix 5: 1H-1H COSY spectrum of of VG-3

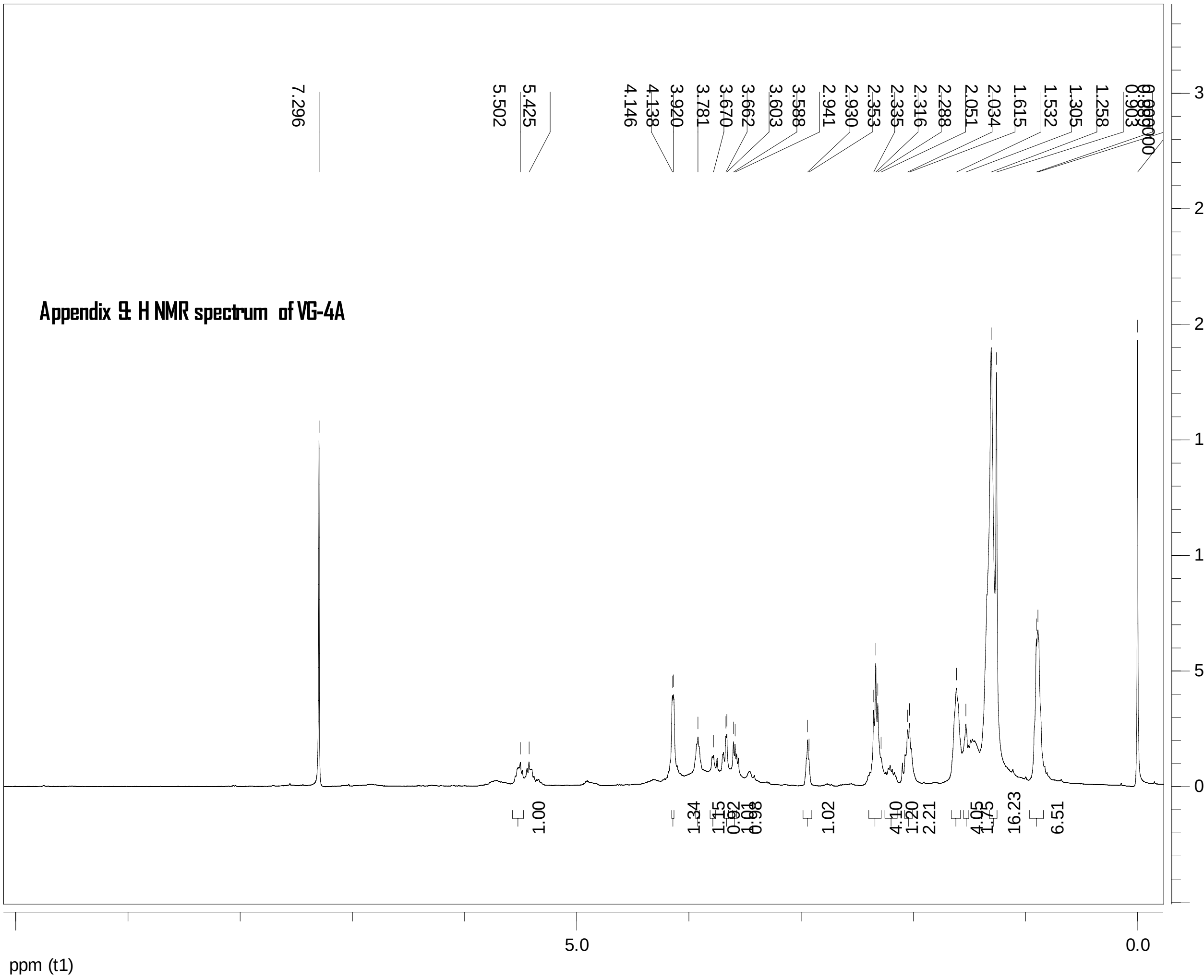


Appendix 6: The HSQC spectrum of VG-3

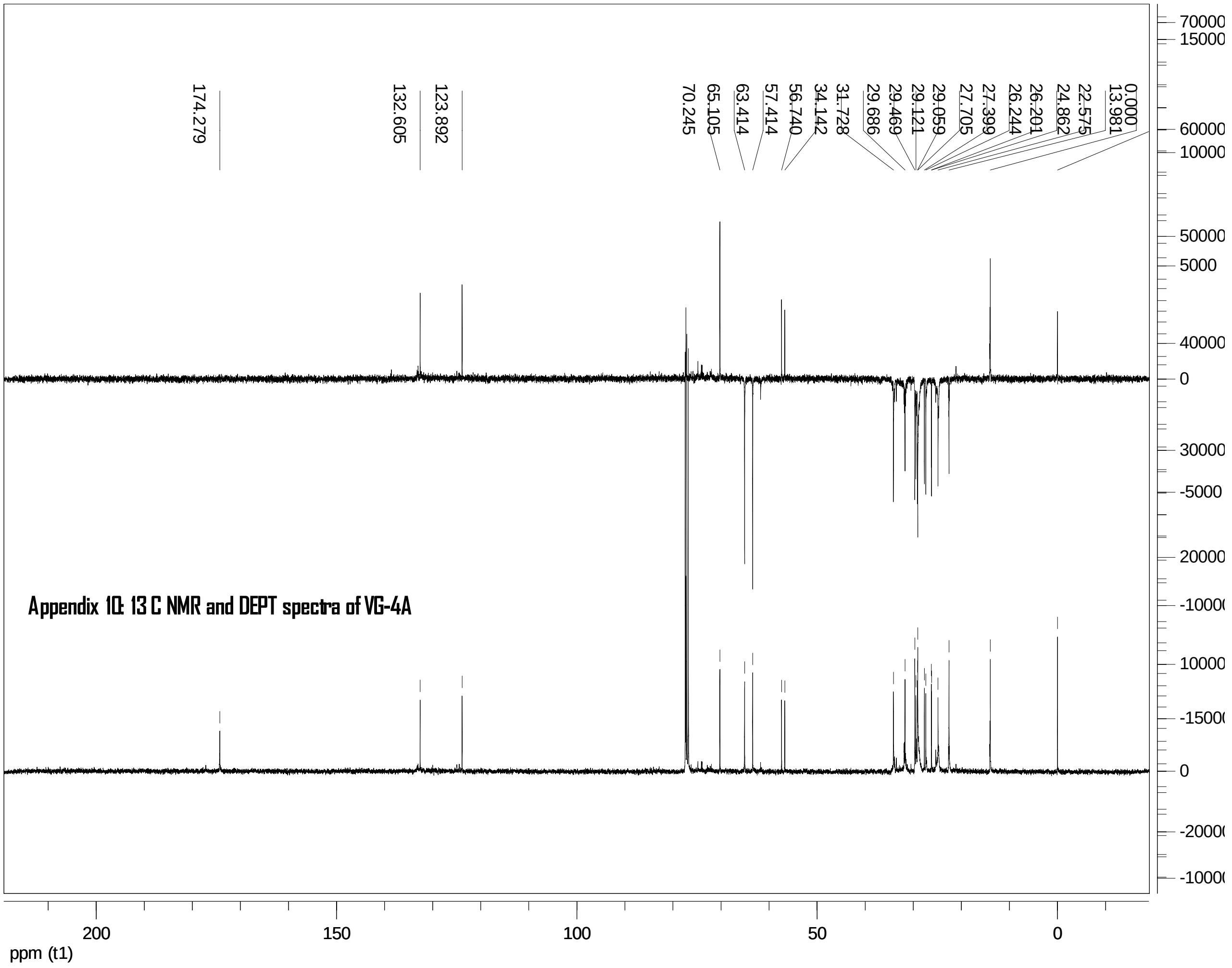

Appendix 7: HMBC Spectrum of VG-3
50
100
150
ppm (t1
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)



Appenndix 9: IR spectrum of VG-4A



Appendix 9: H NMR spectrum of VG-4A



Appendix 10: 13 C NMR and DEPT spectra of VG-4A

DECLARATION

I, undersigned, declare that this project is my original work and has not been presented for a degree in any other university and that all the sources of materials used for this project has been duly acknowledged.

Name: Belete Bedemo

Signature: ____________

This project has been submitted for examination with my approval as university advisor.

Name: Dr. Ashebir Fiseha

Signature: _______________

Place and date of submission: Department of Chemistry
Addis Ababa University
July 2006