

**ADDIS ABABA UNIVERSITY
DEPARTMENT OF CHEMISTRY**



**CHEMICAL INVESTIGATION ON THE
ESSENTIAL OIL OF ARTEMISIA SCHIMPERI**

**GRADUATE PROJECT
(CHEM 774)**

**BY
BERHANU TEFAYE**

MARCH 2007

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ESSENTIAL OIL OF ARTEMISIA SCHIMPERI**

**A GRADUATE PROJECT SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE IN CHEMISTRY**

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Dedicated to

my family : Yodit, Rejik and Kidus

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ABSTRACT

Chemical Investigation on the Essential Oil of *Artemisia Schimperi*

By

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A study of the essential oil of Artemisia schimperi was undertaken. The volatile constituents of the aerial parts of Artemisia schimperi have been examined by GC, ^1H -NMR and ^{13}C -NMR and 18 components comprising 87.4% of the oil have been identified by GC using Kovat's index and in some cases by comparison with standard samples. The oil contained as main components: p-cymene 19.6%, Artemisyl acetate, 12.7%, Geranial 10.5%, Artemisia alcohol 9.2%, and Limonene 6.6%. The oxygenated compounds constitute 63.6% of the oil. One compound was isolated and the structure was elucidated based on spectroscopic technique and by comparison of the data with the reported in the literature.

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1. INTRODUCTION

1.1 Essential oils

It has been known for many centuries that the flowers, fruits, leaves and roots of many plants contain volatile odoriferous substances called essential oils [1]. An essential oil is a mixture of fragrant, volatile compounds, named after the aromatic plant material of a single type and identity from which it has been derived by a physical process and whose odor it has[2].

Essential oils are used in perfumery, aroma therapy, cosmetics, incense, for flavoring food and drink, and to a lesser extent, in medicine and household [3,4]. Essential oil is also known as volatile oil and ethereal oil. The term volatile refers to the fact that this class of oils can be entirely volatilized without change where as the fixed or fatty oils make a permanent greasy mark if placed on a piece of paper [5]. It may also be referred to as “oil of “the raw plant material from which it was extracted, such as oil of clove. The term essential is intended to indicate that the oil is the fragrant essence of the plant from which it is extracted and not in the more common sense of being indispensable. Many oils that are used medicinally are essential oils, which are distilled rather than pressed or otherwise extracted. Medical properties claimed by those who sell medicinal oils vary from skin treatments to remedies for cancer and are often based on historical use of these oils for these purposes. Such claims are now subject to regulation in most countries and have grown correspondingly more vague, to stay with in these regulations. Interest in such uses of essential oils has enjoyed a revival in recent decades, with the popularity of aroma therapy, in which oils are heated and volatilized [6].

The use of essential oil plants for their pleasant fragrance is as old as human civilization. Incense and myrrh are the oldest known aromatic materials; they are mentioned in Assyrian clay tablets [2].

1.2 Methods of Essential oil production

Four processes are used to produce essential oils: solvent extraction, enfleurage expression and distillation.

1.2.1 Solvent Extraction. Solvent extraction is an industrial process in which highly purified, volatile hydrocarbons are used to dissolve aroma compounds from plant material, followed by the removal of the solvent by distillation. The solvent used should be carefully selected. It should quickly dissolve the odoriferous compounds, yet as little as possible of inert matter such as waxes, pigments and albumen. Frequently used solvents are petroleum ether, benzene and alcohol while for the extraction of spices hexane and dichloromethane are commonly applied. Solvent extraction is generally a more costly process than water or steam distillation.

1.2.2 Enfleurage is one of the oldest techniques employed to capture the true odor of the most delicate flowers. This technique originated in Asia where it was a common household practice to place fresh flowers in fat or oil to capture their fragrance. The enfleurage process is mainly advantageous for flowers that continue to produce aroma compounds for several days after they have been picked. Jasmine for example, produces 4-5 times more essential oil than is present at any time in the fresh flower. Using enfleurage, the yield of essential oil from the flowers is higher than when using extraction process with volatile solvents or distillation. Currently the technique has almost died out because of the very high labor cost involved.

1.2.3 Expression is used to obtain the essential oils from the peel of the citrus fruits. E.g. Bergamot, grape fruit, lemon, lime orange, tangerine. It was originally a house hold industry using only very simple tools, but it has been superseded by large-scale industrial processes. Most citrus peel oils are produced as by-products of the citrus juice industry. Many components of the essential oils from citrus fruits are delicate and suffer significantly from heat degradation when exposed to steam distillation. A cold 'expression' process is used to obtain essential oils from citrus fruits.

1.2.4 Distillation: several forms of distillation are applied to produce essential oils, the most important being water distillation, steam distillation and hydro diffusion. Water distillation or hydro distillation is a very old process for the production of essential oil and has undergone centuries of improvement.

Steam distillation is a similar process, but a hot steam is forced through a plant material to extract the essential oil. Hydro diffusion is a recent process in which low-temperature; low- pressure steam is used to extract the essential oils.

The principle of water distillation is to boil and vaporize a suspension of aromatic plant material and water in a vat so that its vapors can be condensed and collected. The essential oil which is immiscible with water is then separated by gravity in a 'Florentine flask'. The water in the still must be kept in motion to prevent the plant material from clogging together and settling at the bottom of the still. This would result in a low yield of essential oil, charring of the plant material and degradation of thermo-instable compounds, resulting in still odors.

In steam distillation (sometimes called 'dry steam distillation') a separate steam generator is attached to the still. The plant material is placed on a grid in the distillation vat, but no water is added. Steam produced in the generator is forced through the material to be distilled. High pressure steam is often used e.g. steam of 5-10 bar pressure at 150-200° C.

Hydro diffusion is a distillation method developed in the 1980s. Low pressure steam (<0.1 bar) is used and the volatile components are extracted from the plant material mainly by osmosis. In this method a distillation vat is filled with comminuted plant material. Steam is fed to the still but, unlike steam distillation, it is fed from the top of the still and moves downwards through the plant material by gravity. After passing through the plant material, the steam and volatile compounds flow through the condenser placed at the bottom of the still and are collected in an oil separator. Hydro diffusion has shown excellent results under experimental conditions: short distillation times, low steam consumption, high yields of high-quality oil and absence of high temperatures [2].

1.3 Composition of Essential oils

Essential oils are complex mixtures of sometimes hundreds of chemical compounds. Most of these compounds can be grouped in to a few major classes, but there are also many components of essential oils that bear little resemblances to these classes. The compounds are classified in to four major groups: aliphatic compounds, terpenes, and terpene derivatives, benzene derivatives and miscellaneous compounds [2].

1.3.1 Aliphatic compounds

Aliphatic hydrocarbons occur abundantly in food stuffs such as fruit, but contribute to their odor to a limited extent only. Aliphatic esters are important flavor and fragrance compounds occurring widely in nature.

1.3.2 Terpenes and terpene derivatives

Although terpenes show a wide structural diversity, they share a common characteristic: They are built from two (monoterpenes), three (sesquiterpenes) or more isoprene (C_5H_8) units. Isoprene is one of the basic compounds in animal and plant biochemistry from which carotenoids, steroids, and rubber are also formed. It is formed from acetylCoA that plays a role in the synthesis and oxidation of sugars. The terpene hydrocarbons contribute

to the odor and taste of essential oils to a limited extent only, but their oxygenated derivatives are among the most important aroma chemicals. In lemon, orange and other citrus-peel oils about 95 percent of the essential oil consist of terpenes [7].

Monoterpenes conform to the molecular formula $C_{10}H_{16}$ and can be acyclic, monocyclic or bicyclic. There are even a few tricyclic monoterpenes: cyclofenchene and tricyclene. Acyclic mono terpenes are relatively unstable and some have a slightly aggressive odor, because of their strongly unsaturated structure. E.g. myrcene and ocimene. Cyclic mono terpenes occur in essential oils, sometimes in considerable amounts. By themselves they generally contribute relatively little to the odor of a fragrance or flavor product, but often serve as starting materials for the biological or chemical synthesis of flavor and fragrance compounds e.g. α -terpinene, γ -terpinene, para- menthadiene, or limonene.

Sesquiterpenes are compounds generated from three isoprene units and conforming to the formula $C_{15}H_{24}$. Many sesquiterpenes are bi-cyclic having two C_6 rings or a C_6 and a C_5 ring.

Oxygenated derivatives of monoterpenes and sesquiterpenes are more important than the terpene hydrocarbons as aroma chemicals. The characteristic odor of many essential oil is representative of the combined odors of the oxygenated compounds. Important groups of oxygenated compounds are alcohols, aldehydes ethers, ketones, acids and esters.

Acyclic monoterpene alcohols and acyclic sesquiterpene alcohols occur in many essential oils and contribute strongly to their characteristic odor. Some of them such as citronellol, geraniol, linalool and nerol are also synthesized from turpentine on an industrial scale.

The most important aldehydes derived from acyclic monoterpenes and sesquiterpenes are citral and citronellal.

Esters of terpene alcohols and lower fatty acids, in particular acetates are highly important as flavor as well as fragrance materials.

1.3.3 Benzene derivatives: This group includes a very large and varied group that includes many natural and synthetic flavor and fragrance compounds. The most important hydrocarbon derived from benzene is para-cymene; it occurs in many essential oils and has a weak citrus odor when pure. Of the benzenoid alcohols and aldehydes important compounds of essential oils are phenyl ethyl alcohol, cinnamic alcohol, and cinnamic aldehyde.

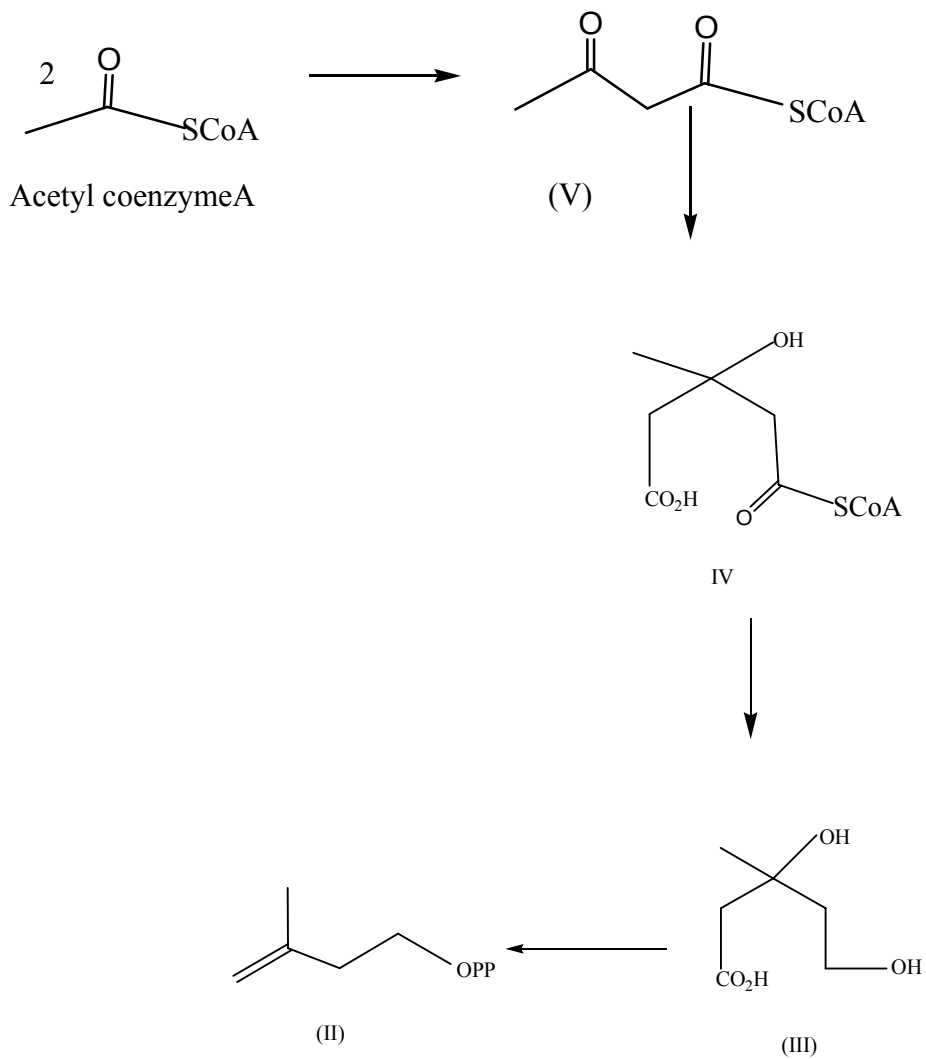
1.3.4 Miscellaneous compounds several nitrogen and sulphur compounds impart characteristic sensory properties to essential oils. For example allyl isothiocyanate found in mustard oil [1]. Isolated or synthetic nitrogen compounds are used in industry to modify jasmine oil [2].

1.4 Biosynthesis of the constituents of Essential oil

The function of terpenes in plants and other organisms is not clear [8] although they sometimes possess toxic properties linked to the protection of the species. Terpenes are products of secondary metabolism. The terpenoids comprise the largest group of natural products and over twenty thousand such structures have been described from plant sources. They are derived biogenetically from the five carbon precursor isoprene hence isoprenoids [9].

1.4.1 Biogenesis of Isopentenyl pyrophosphate

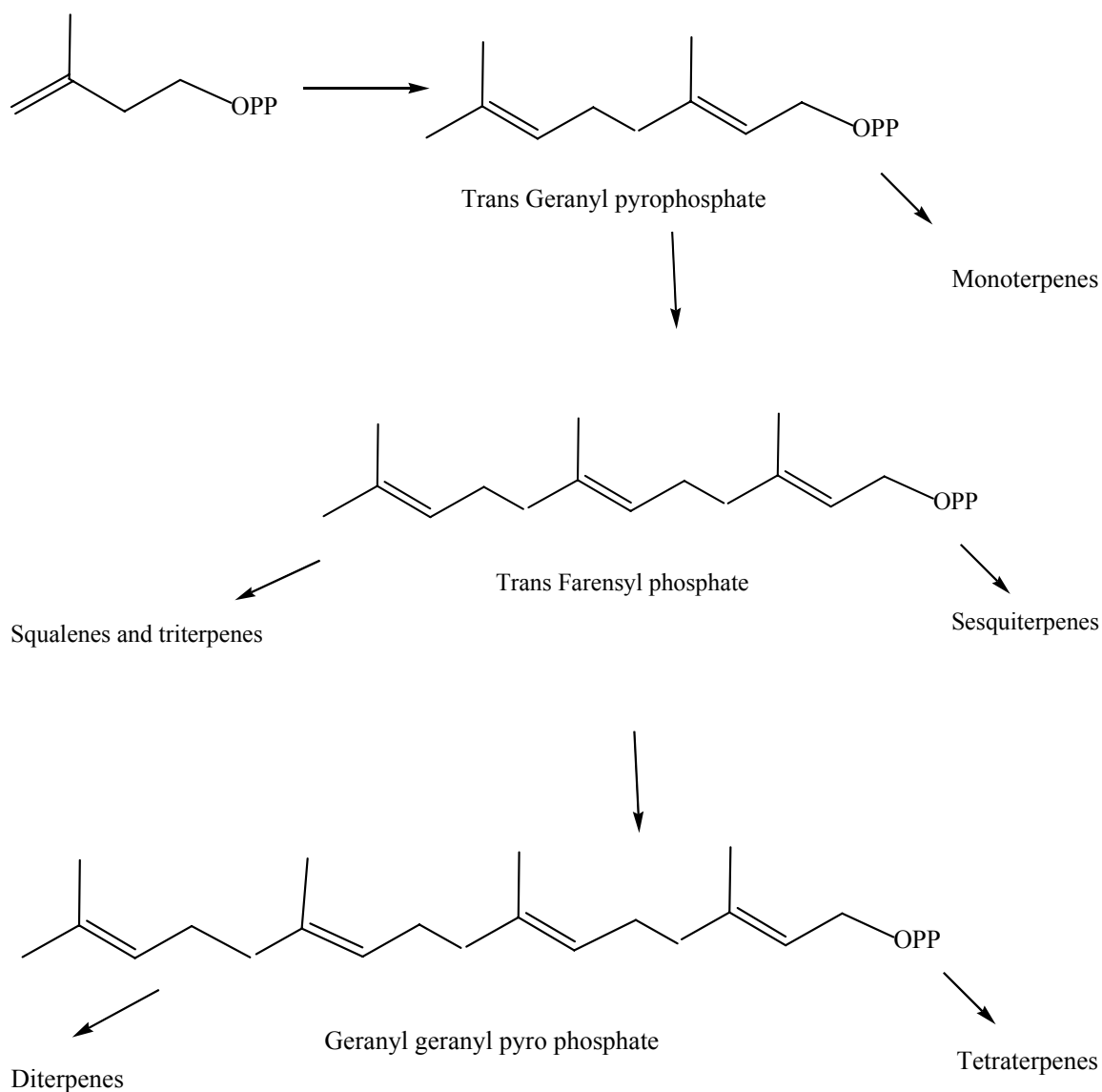
The key building block, isopentenyl pyrophosphate (II) arises from mevalonic acid (III) via hydroxymethyl glutarate (IV) [10]. The starting point of this metabolic pathway is believed to be the condensation of two molecules of acetylcoenzymeA to form acetoacetyl coenzyme A (V). Isopentenyl pyrophosphate (II) has two reactive sites and can polymerize in a variety ways to form larger terpenes. Its head to tail dimerization is the most commonly encountered process, although head to head and tail to tail linkages are also found. The multitude of structural types in terpenes arises from intramolecular rearrangements of several basic skeletons formed by the cyclization of the linear precursors shown in [scheme 3](#) where opp represents oxygen-alkylated pyrophosphate.



Scheme 1: Biogenesis of isopentenyl pyrophosphate

1.4.2 Biogenesis of terpenoids

Isopentenyl pyrophosphate is readily converted to terpenes by polymerization in a variety of ways as shown in scheme 2 [11].

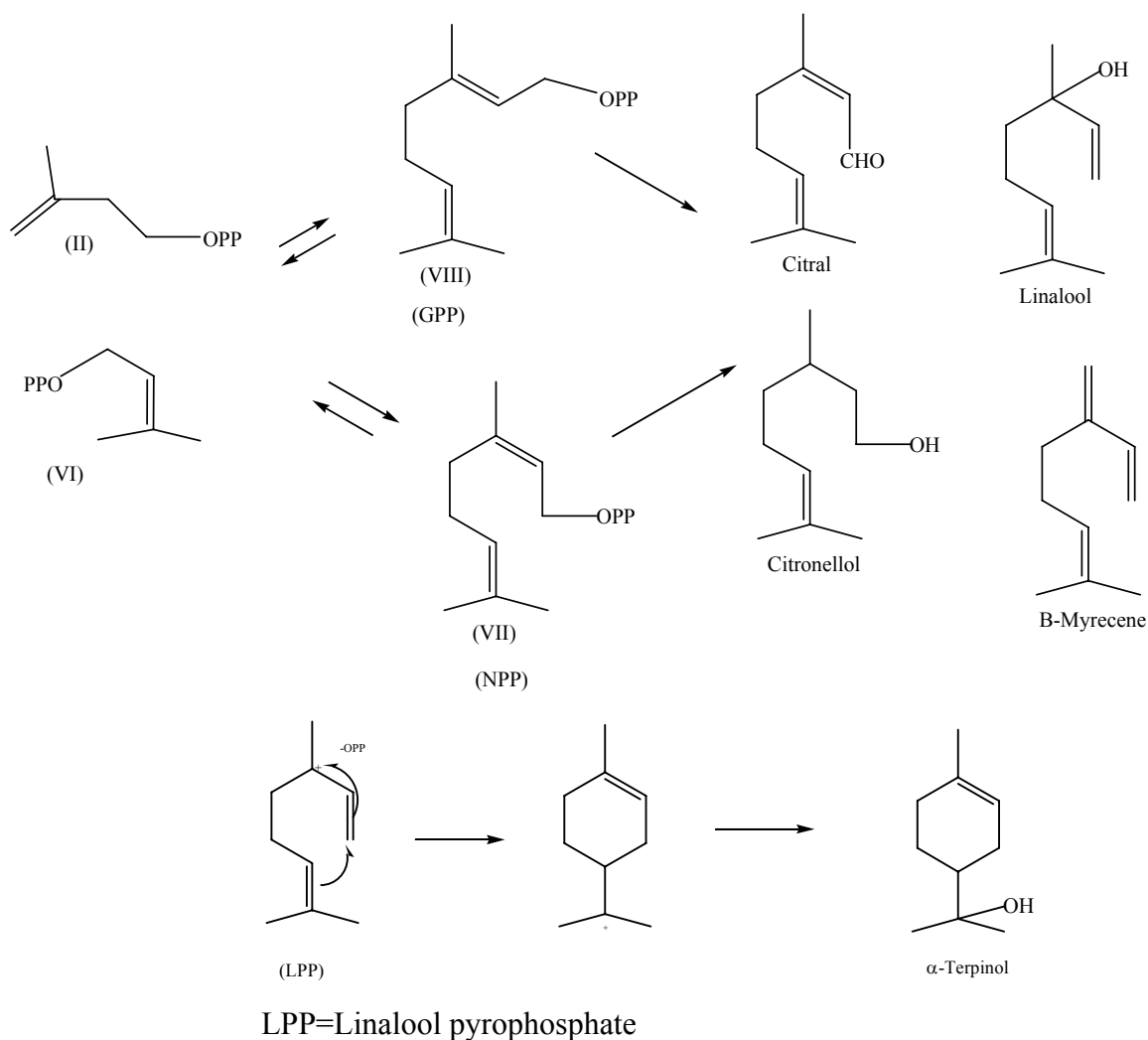


Scheme 2 Biogenesis of Terpenoids

1.4.2.1 Biogenesis of monoterpenes

Monoterpenes which are presumed to be derived from geranyl pyrophosphate have been known for hundreds of years as components of essential oils of higher plants [12].

The first stage in the formation of mono terpenes is the linking of isopentenyl pyrophosphate(II) and dimethylallyl pyrophosphate(VI) to give neryl pyrophosphate(VII) or geranylpyro phosphate(VIII):scheme (3) [13].

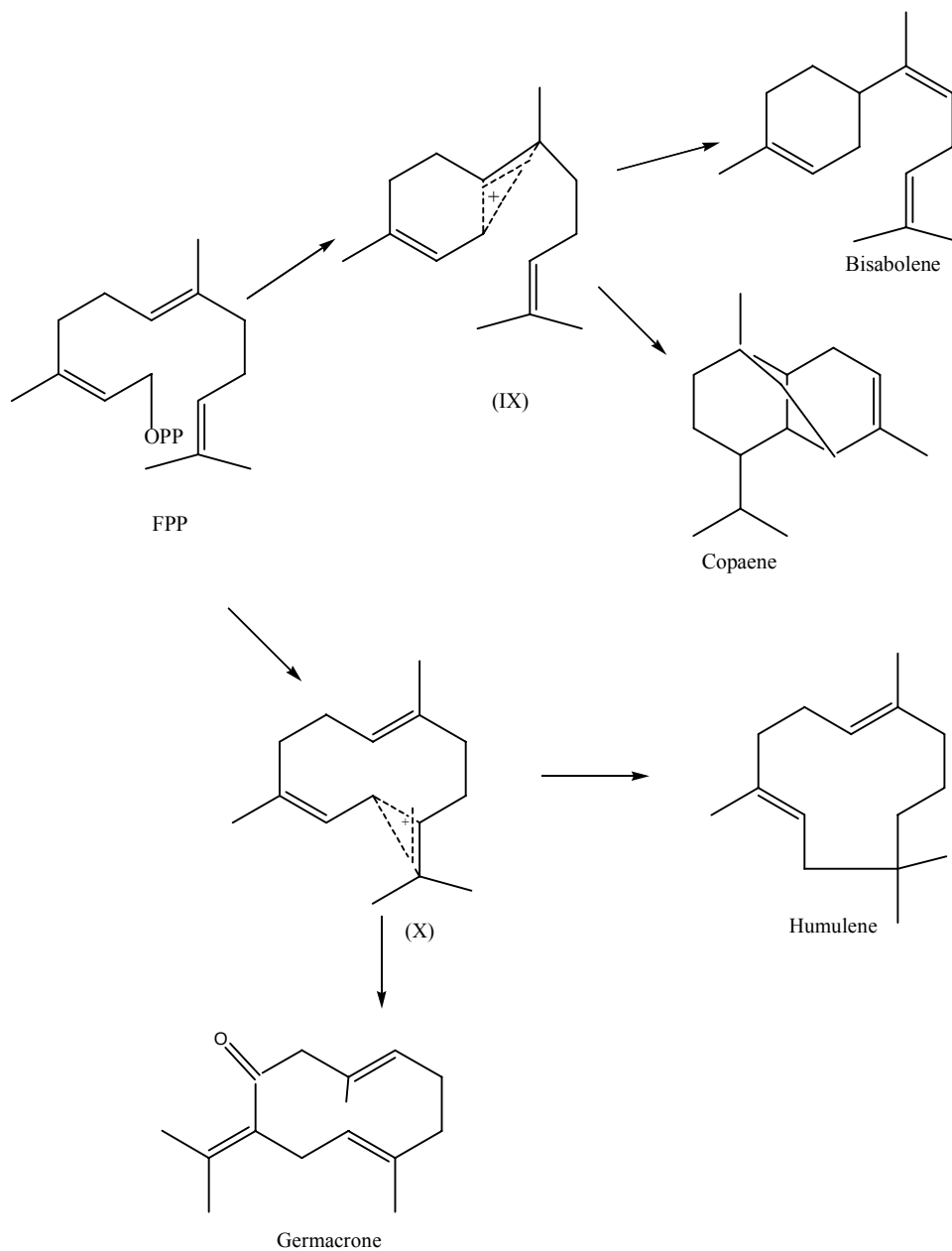


Scheme 3: Biogenesis of monoterpenes

1.4.2.2 Biogenesis of sesquiterpenes

Sesquiterpenes are C_{15} hydrocarbons and their oxygenated analogues. As a family, the sesquiterpenes encompass an almost bewildering array of structural types and more than fifty basic skeletons have been recognized. They arise from the cyclization of farnesyl

pyrophosphate (FPP) and subsequent rearrangements of the resulting carbonium ions [8, 14]. (Scheme 4).



Scheme 4: Biogenesis of sesquiterpenes

1.5 The Genus *Artemisia*

Artemisia is a large, diverse genus of plants with between 200-400 species belonging to the daisy family Asteraceae which is one of the largest families of vascular plants [15]. It comprises hardy herbs and shrubs known for their volatile oils. They grow in temperate climates of the northern hemisphere and southern hemisphere, usually in dry or semi-dry habitats. The fern like leaves of many species are covered with white hairs. The aromatic leaves of many species of *Artemisia* are medicinal, and some are used for flavoring. Most species have an extremely bitter taste. *A. dracunculus* (Tarragon) is widely used as herb, particularly important in French Cuisine [16]. *A. scoparia* is purgative and is also used for treating ear ache and the smoke for treating burns [17]. *A. absinthium* is anti spasmodic, and also used for the treatment of cough, malaria, [18] and also to make absinthe liqueur [16]. *A. siversiana* shows anti microbial activity [19], and *A. annua* has anti malarial properties [19]. The leaves of *A. afra* are used for the treatment of cold, constipation, cough and gout [20] *A. abyssinica* is used for the treatment of STDs, leprosy [21] and tonsillitis [18]. *A. douglassiana* shows anti tumor activities [9].

The Genus *Artemisia* (Asteraceae) is a rich source of sesquiterpenoids which are receiving renewed phytochemical attention due to the biological and chemical diversity [19]. The essential oils of many *artemisia* species have been investigated before (Table 1).

Table 1 Constituents of Essential oil of some Artemisia Species

Compound	A Annua	A.douglasiana	A.vallesiaca	A.persica	A.nilagirica	A.Sieberi	A.afra	A.vulgaris	A.Austria ca	A.spicegera	A.herb- alba	A.abys sinica	A.rehan
α -terpinol		+							+		+		
Camphor		+	+		+	+			+	+	+		+
1,8-cinole			+	+	+		+		+	+	+	+	
α - pinene									+				
Terpinen-4-ol									+		+		+
Camphene			+	+					+	+			
Thymol										+			
β - Thujone		+											
Sabinene		+					+	+					
α - thujone							+	+					
A. Ketone	+	+					+				+		
A. Alcohol	+	+					+				+		
Sabinene		+											
Borneol		+	+		+				+	+	+		
Davanone				+									+
Artemone				+									
P-cymene				+			+						
A.alcohol acetate							+					+	
Yomogi alcohol							+					+	
reference	[22]	[22]	[22]	[22]	[22]	[22]	[22] [25]	[22]	[22]	[22]	[22]	[25]	[25]

In Ethiopia there are only four species namely *A. afra*, *A.absinthium*, *A.abysinica* and *A.schimperi* belonging to this large and well investigated genus which has proved a variety of regular as well as irregular monoterpenes and sesquiterpene lactones [23].

1.5.1 *Artemisia afra*: Chirakot (Gur), Chikugn (Amh). Commonly known as African wormwood. *Artemisia afra* is one of the oldest and best known medicinal plants, and is still used effectively today in South Africa by people of all cultures. The list of uses covers a wide range of ailments from coughs, colds, fever, and loss of appetite, colic, head ache, ear ache, and intestinal worms to malaria. *Artemisia afra* is used in many different ways and one of the most common practices is to insert fresh leaves in to the nostrils to clear blocked nasal passages. Another may be not so common use is to place leaves in socks for sweaty feet. The roots, stems and leaves are used in many ways and taken as enemas, poultices, infusions, body washes, lotions, smoked, snuffed or drunk as tea. *A. afra* has a very bitter taste and is usually sweetened with sugar or honey when drunk [20].The essential oil of *A. afra* has been investigated before by African Laboratory for Natural Products (ALNAP). *Artemisia* alcohol acetate, yomogi alcohol, geraniol and bornyl acetate were found to be the major components [25].

1.5.2 *Artemisia absinthium*: Ariti (Amh)

Artemisia absinthium (*Artemisia rehan*) locally known as “ariti” is a perennial odorous herb which is widespread in and native to Ethiopia. It is a tradition for Ethiopian women to carry large bunches of this plant to church, because the aromatic scent helps prevent feeling of drowsiness. Abegaz and Paulos identified 22 components in the oil of *A.rehan* using GC, UV, and ¹H NMR with Davanone (44.36%) and camphor (23.49%) as major components [24]. The composition of the oil is also reported by ALNAP [25].

1.5.3 *Artemisia abyssinica* Schultz-Bip Chikugn(Amh) : *A. Abyssinica* is endemic to Ethiopia[20].. The oil contains the irregular monoterpenes yomogi alcohol (32%) and *Artemisia* alcohol acetate (27%) as its major constituents. The composition of the oil is also reported by ALNAP [25].

1.5.4 *Artemisia schimperi*

It is an endemic perennial with yellow flowers and fragrant leaves, which is found in the Bale and Gondar mountains of Ethiopia at Altitudes of 3600-4200m [23]. Annual or short lived bushy herb 30-40cm high, strongly aromatic, stems usually several and radiating from the root apex [26]. The essential oil has not been analyzed before, but Abegaz B. et al have reported 1,5-octadien-3-hydroxy-3-methyl-7-one, artemisylacetate, linalyl acetate and quercetin 3,3',4'-trimethyl ether as constituents of the plant [23].

2. Objective

The main objective of this project was to identify the components of the essential oil of *Artemisia schimperi* and determine their percentage composition using GC and, to isolate and characterize the components of the oil using Column chromatography and spectroscopic techniques.

3. Results and discussion

Hydro distillation of the aerial parts of *A. schimperi* yielded 1.2% (v/w) of the essential oil. The essential oil obtained from *A. schimperi* was analysed by GC. Constituents of the oil were identified using Kovat's index

The oil derived from *A. schimperi* constitutes about 40 components; 18 were identified. The identified components account for 87.4% of the oil. p-cymene 19.6%, Artemisyl acetate 12.7%, Geranial 10.5% Artemisia alcohol 9.2%, and Limonene 6.6% were the major components of the oil.

The oil was subjected to column chromatography and fractionated into hydrocarbon and oxygenated fractions. Compound F-2 was isolated by column chromatography from the

oxygenated fraction and its structure was elucidated using spectroscopic data. The compound was identified to be Artemisyl acetate

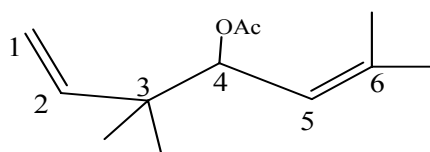
Table 2: compounds identified from the essential oil of *A. schimperi* and their Chemical composition

RT* on EC-5,min	KI**[27]	Compound	Percentage	Methods of Identification
7.10	ND***	α -pinene	5.0	STD Θ
9.21	973	sabinene	1.3	KI
10.02	ND***	p-cymene	19.6	STD Θ
11.25	1034	limonene	6.6	KI
11.55	1042	z-ocimene	1.0	KI
12.24	1062	artemisia ketone	2.3	KI
12.58	1071	z-Linalool Oxide	2.3	KI
13.07	1085	artemisia alcohol	9.2	KI
15.93	1163	borneol	2.4	KI
16.34	1174	artemisiyl acetate	12.7	NMR, IR
17.09	1194	α -terpineol	0.8	KI
19.46	1259	β -phenethyl acetate	6.0	KI
20.08	1276	geranial	10.5	KI
20.67	1292	1,3-p-menthadien-1-al	1.9	KI
21.43	1314	cinnamyl alcohol	1.0	KI
21.75	1323	methyl decanoate	2.0	KI
28.05	1510	methyl Laurate	1.1	KI
29.85	1567	isopropyl benzoate	1.2	KI

*=Retention time **=Kovat's index, ***= Not determined, Θ =comparison with RT of standard samples

The oil contains Artemisia ketone, Artemisia alcohol and Artemisyl acetate which are also constituents of other essential oils of Artemisia species. Artemisia abyssinica and Artemisia schimperi contain Artemisia acetate as common component. P-cymene and Artemisia acetate are also found in Artemisia afra.

Compound F-2, a fragrant light yellow liquid, which is an irregular mono terpene ester, was obtained from the ethyl acetate fraction of *A. schimperi*. It was identified by comparison of the acquired spectroscopic data with the reported in the literature for the same compound [23]. The IR(neat) spectrum displayed absorption bands at ca 3050 cm^{-1} for unsaturated carbon stretching, at 1737 cm^{-1} for carbonyl of ester and at 1241 cm^{-1} for C-O stretching. The presence of vinyl proton (δ 5.91), two methyl groups on a double bond (δ 1.75), one methyl group (δ 2.03) indicative of acetyl group and two methyl groups (δ 1.01) attached to an sp^3 hybridized carbon was evident from the ^1H NMR (400MHz, CDCl_3) spectrum (Table 3). The ^{13}C NMR spectrum (Table 4) displayed 12 carbon resonances corresponding to 5 primary, 1 secondary, 3 tertiary and 3 quaternary carbon atoms.



F-2

Artemisyl acetate

Table 3: ^1H NMR: Comparison of ^1H NMR spectral data of F-2 with that of Artemisia Acetate [23].

Proton	$\delta(\text{ppm})$ F-2, CDCl_3	$\delta(\text{ppm})$ A.acetate(Literature), CDCl_3
3-Me	1.01	0.98
6-Me	1.75	1.72
4-COMe	2.03	2.01
1-H (trans)	4.98	4.99
1-H (cis)	5.04	5.01
5-H	5.09	5.09
4-H	5.33	5.32
2-H	5.91	5.91

Table 4: ^{13}C NMR: Comparison of ^{13}C NMR spectral data of F-2 with that of A.Acetate [23].

Carbon	δ (ppm), F-2, CDCl_3	δ (ppm),A.Acetate, CDCl_3
1	112.53	112.54
2	120.49	121.04
3	40.87	41.17
3 –Me	18.6	18.83
3 –Me	21.20	21.14
4	76.84	77.20
4 -CO	170.36	170.27
5	144.30	144.79
6	138.26	138.23
6-Me	23.88	24.13
6 –Me	25.99	26.03
Ac	22.05	22.54

4. Summary

Chemical investigation on the essential oil of *A. schimperi* was conducted. Identification of the constituents was done using ^1H NMR, ^{13}C NMR, and GC. The

investigation indicated that the oil has the irregular monoterpenes Artemisyl acetate, Artemisia ketone and Artemisia Alcohol which are also present in other Artemisia species.

5. Experimental

^1H NMR: Bruker (400MHz, CDCl_3), δ values are given in ppm

^{13}C NMR: Bruker (100 MHz, CDCl_3)

GC: Dani 1000, fused silica capillary column coated with EC-5

Column length (30m), internal diameter (0.32mm) Film thickness (0.25micron)

Stationary phase: 5% phenyl 95% methyl poly siloxane

Column temperature: Initial temp. was held at 50°C for 5 min, then programmed to 240 °C at 4 °C/min.

Carrier gas: Nitrogen at a flow rate of 1 ml/min.

FID temperature: 260 °C

Injection temperature: 210 °C

Split injection ratio: 1:80

IR: FT IR Perkin Elmer Spectrum BX

Chromatography

Analytical TLC: silica gel 60 F₂₅₄ (Merck), 20x20 cm pre coated plate.

Column chromatography: silica gel S, 0.032-0.063 mm

Plant material

The plant material was collected 31 Km from Goba towards Dolo at an altitude of 4000m (06°49'28.8''N, 39° 51'86.9''E) by Prof. Sebsebe Demissew, AAU, on 4 Sept, 2004. Voucher specimen is deposited at the National Herbarium, department of Biology, AAU. Collection number 6348. The essential oil was hydro distilled and kept in a sealed vial in the fridge.

Isolation and Characterization

The Essential oil sample was obtained by hydro distillation of the plant. Isolation of the compounds was conducted using column chromatography and gas –liquid chromatography.

1.2 g of the oil was applied on a silica gel (60g) column and eluted successfully with hexane (300ml) and ethyl acetate (250ml) to obtain hydrocarbon and oxygenated fractions respectively. The oxygenated fraction was further fractionated on a silica gel column with 5% ethyl acetate in petroleum ether. A total of 26 fractions each of about 10 ml were collected. Fractions 9-12 afforded 16mg of artemisylacetate (F-2).

Artemisyl acetate(compound F-2)

Light yellow liquid: RT 16.34 min, IRV_{max} cm⁻¹; ca 3050,2971,1737,1446,1369,1241, 970,914; ¹H NMR(400MHz,CDCl₃) δ1.01(6H,s,3-Me),1.75(6H,s,6-Me),2.03(3H,s,4-H),4.98(dd,H-1trans),5.04(dd,H-1cis),5.09(dm,H-5),5.33(d,H-4),5.91(dd,H-2).

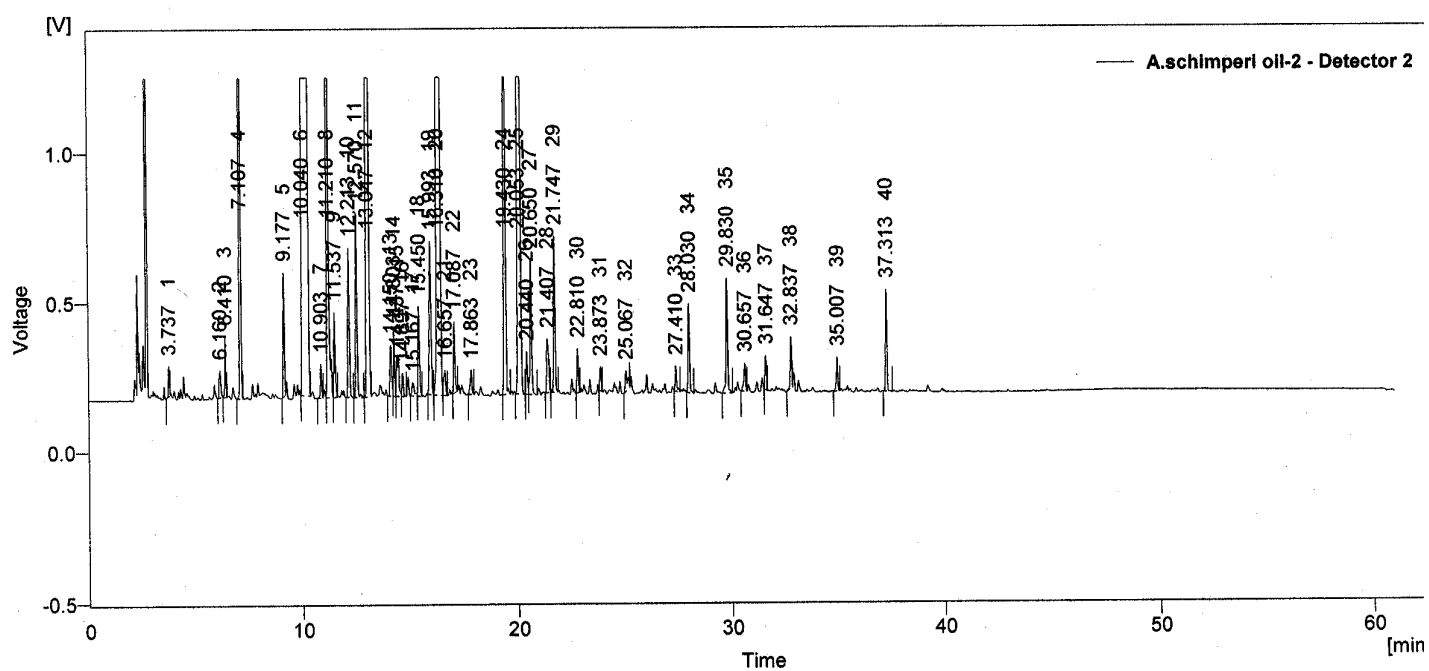
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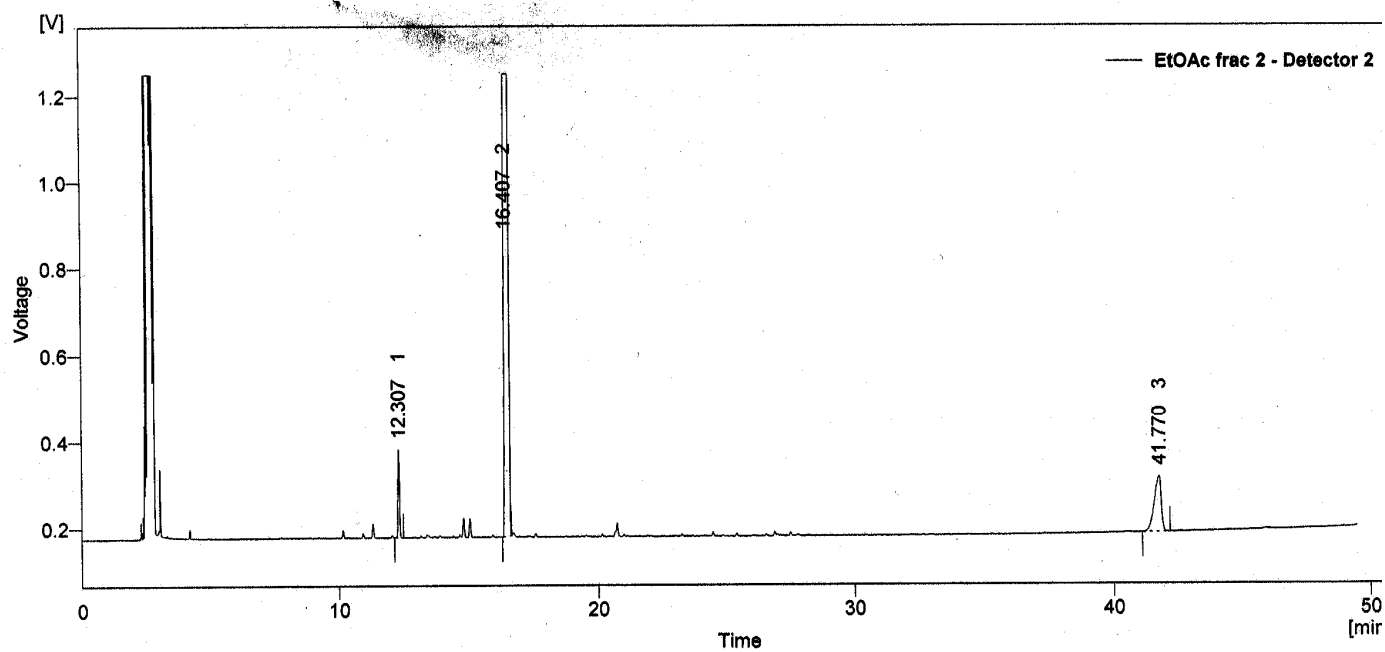
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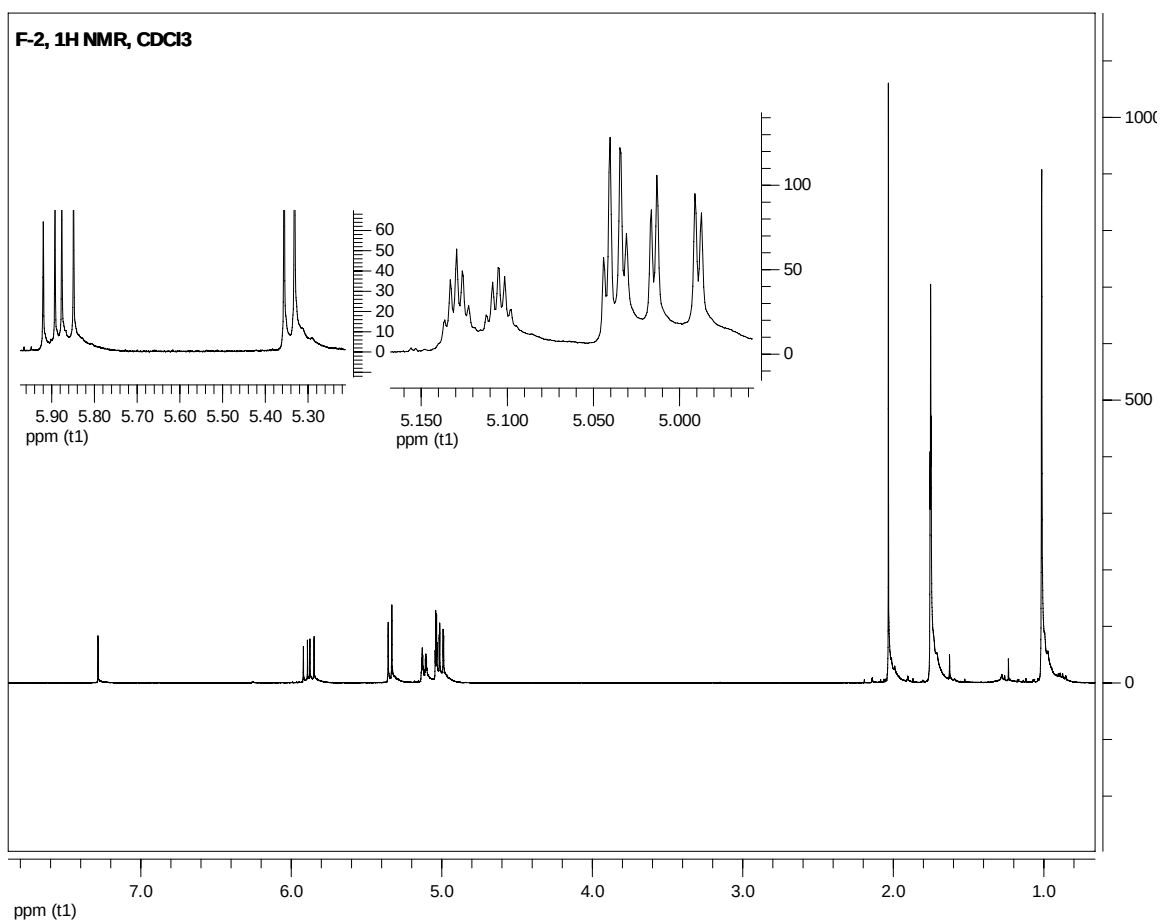
Appendix



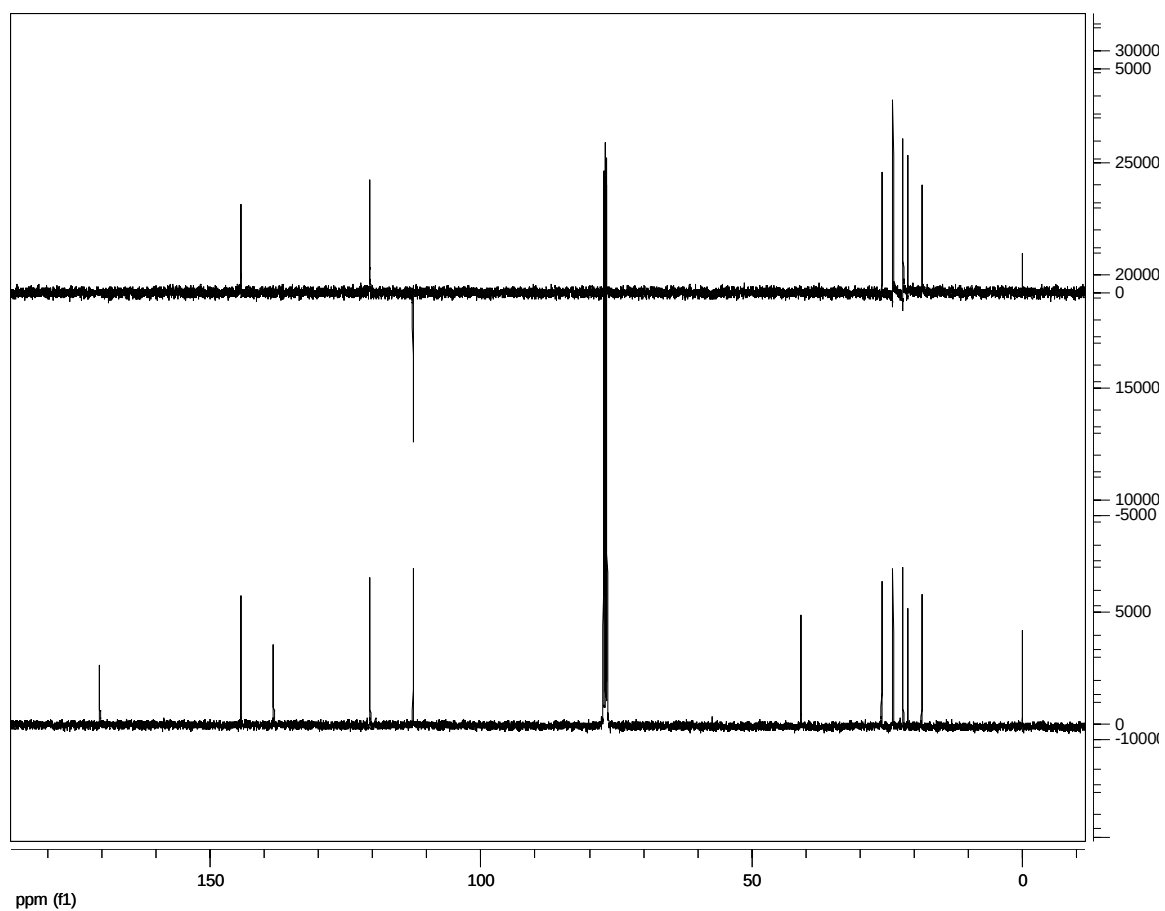
GC of Artemisia schimper oil



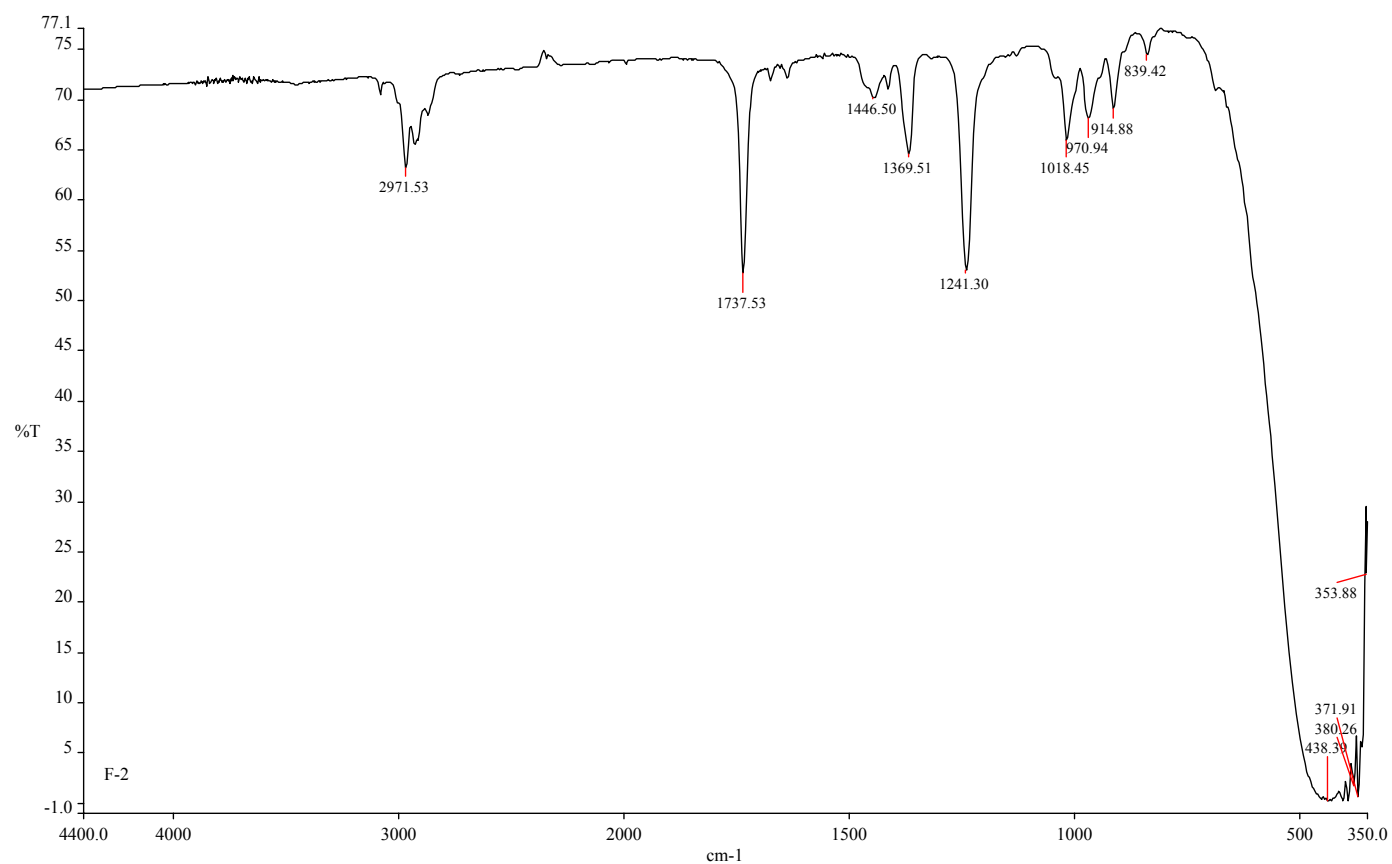
GC of F-2



^1H NMR of F-2



^{13}C and DEPT spectra of F-2



IR spectrum of F-2

