ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCES

Thesis

PHYTOCHEMICAL INVESTIGATION ON LEAVES OF *LEONOTIS OCYMIFOLIA*

B Y: ASEFA MEKONENN

ADVISOR: AMAN DEKEBO (PhD)

July, 2015

PHYTOCHEMICAL INVESTIGATION

ON

LEAVES OF *LEONOTIS OCYMIFOLIA*

A graduate thesis submitted to the school of applied Natural **sciences**

Program: Applied chemistry

BY: Asefa Mekonenn

In partial fulfillment of the Requirement for Master of Science Degree in chemistry (Organic Chemistry)

July, 2015

Adama science and Technology University

As thesis research advisor, I here certify that we have read and evaluated this thesis prepared under my guidance by Asefa Mekonenn entitled phytochemical investigation of leave of *Leonotis ocymifolia.*I recommended that it be submitted as fulfilling the THESIS requirement.

Aman Dekebo (PhD) ---------- ----------

Major advisor Signature date

As Members of the board were examiners. Thesis open defense Examination, we certify that we have read, evaluated the Thesis prepared by Asefa Mekonenn and examined the candidate. We recommend that the Thesis requirement for the Degree of Master of science on Organic chemistry.

_____ _____ _____

Chair person signature Date

_____ _____ _____

Internal Examiner Signature Date

_____ _____ _____

External examiner Signature Date

Acknowledgement

I would like to thank my advisor Dr. Aman Dekebo for his valuable guidance, advice, encouragement, support, and critical comments on this research work from the beginning up to the completion of this study.

I am thankful to my family Mukria Shefraw, Henok Dide, Abayin Kasahun, Salamon Ganen and Darje Hailu for their support and encouragement. My deepest gratitude also goes to Tsefaye Tashome who helped me in material I needed for the study..

I am grateful to Getachew Shanqi cooperation for giving me information on samples collection, and facilitating transportation to sample site.

I would also like to thank those Laboratory worker specially W/t Mariyama Kufa for their kind guidance in the lab.

TABLE OF CONTENTS

Contents **Page**

Acknowledgements……..……..i

Table of Contents…….…..……ii

List of Tables……..…….…….iv

List of Figure……..……..v

List of Schemes…….……...…vi

List of Appendices……..……..…vii

Abbreviations ……..……...viii

Abstract……..……....xi

1 Introduction 1

1.1 Justification of this study 2

1.2 Objectives of the study…… 2

1.1.1 General Objective 2

1.1.2 Specific Objectives…… 2

2. Literature Review 3

2.1 *Leonotis ocymifolia*.....…….....3

2.2 Geographical distribution……..…….…… 3

2.3 Medicinal application of *Leonotis* species…… 4

2.4 Chemical composition of *Leonotis ocymiflia*…… 4

3 Terpenes 8

3.1 General structure…… 8

3.2 Biosynthesis..... 9

3.2.1 Sesquiterpenes…… 9

3.2.1 Diterpenes……..…….....10

4 Materials and Methods.....13

4.1 Plant material…..…… 13

4.2 Chemicals and instruments.....13

4.3 Extraction…….…….....14

4.4 Coding system…….....15

4.5 Thin layer chromatography analysis…….....15

4.6 Isolation of chloroform extracted using CC… 15

4.7 Phytochemical test…….....…….16

4.7.1 Detection of alkaloids…….....16

4.7.2 Detection of saponins.....……...16

4.7.3 Detection of tannins……..……...……...16

4.7.4 Detection of terpenoids.....16

5. Results and discussion ……..……...…17

5.1 Characterization of compound L.O-1…….17

6 Conclusions and Recommendation……...…….19

References.....20

List of Tables

Table-1 The secondary metabolites identified from *Leonotis* plants……..…....5

Table-2 Extraction……..……..……14

Table-3 Isolation of chloroform extract using CC…….……..……..…….…...15

Table-4 PTLC Profile of F_{18} ……...…….....16

Table-5 Phytochemical tests……..……..….16

Table-6 Proton Decoupled ^{13}C NMR and DEPT spectral data of compound **LO-1**.....18

Table-7 The ^{1}H NMR spectral data of compound **LO-1**…….....…….……18

List of Figure

Figure-1 Picture of the plant taken by the author……..…….3
Figure-2 Diterpene lactone reported from leaves of *Leonotis ocymifolia*……..……...……4
Figure-3 Different derivates of morrubin…….……..…….4
Figure-4 Some structures of compounds isolated from *Leonotis* species……..…..……7
Figure-5 Isolation of compound from chloroform extract by CC…….…..…….……..14
Figure-6 The proposed structure of **LO-1**……..…..…….……..19

List of Schemes

Scheme-1 Parent hydrocarbons of terpene (isoprenoids)…..……..…….……8

Scheme-2 Biogenesis of sesquiterpene……...…….……..10

Scheme-3 Some common plant diterpene……..……...…….11

Scheme-4 Biosynthesis of diterpene…….12

Scheme-5 Extraction……..14

Appendices

Appendix -1 UV-spectrum of **LO-1**……..…….……23

Appendix -2 IR spectrum of **LO-1**……..……...24

Appendix -3 ^{1}H NMR spectrum of **LO-1**……...……..……25

Appendix -4 ^{13}C NMR spectrum of **LO-1**.....26

Appendix -5 DEPT-135 of **LO-1**……..……...……27

Abbreviations

CC……column chromatography

^{o}C……..Degree Celsius

DEPT……Distortion Enhancement by Polarization Transfer

IR…….Infrared

NMR……Nuclear Magnetic Resonance

PTLC.....Preparative Thin Layer Chromatography

ppm……...Parts per million

Rf……...retention factor

TLC…….....Thin Layer Chromatography

TMS……...Tetra methyl silane

UV…….ultra violet

WHO…….World Health Organization

δ……the symbol used to indicate chemical shift value

Abstract

Leonotis ocymifolia belongs to the family Lamiaceae, in which the genus contains about 30 species. It is an indigenous to Eastern and Southern Africa, where people used locally for treatment of fever, intestinal parasites and headache. But, no detailed scientific investigation is available on the chemical composition of this plant. So, this research was aimed to isolate and characterize the chemical constituents from leaves of the plant. To achieve this objective, the leaves of the plant was extracted successively with petroleum ether, chloroform, chloroform/methanol, methanol. Phytochemical screenig was carried out on chloroform extract. The result indicated the presence of alkaloid, taninns, terpenoid and absence of sponnins. The chloroform extract was selected for further fractionation and prufication and subjected to column chromatography. The column was eluted with petroleum ether, methanol, chloroform with increasing polrity. Based on PTLC profile and one pure compound was obtaned from fr18 coded as LO-1 was characterized as1,2,3,5,6,7,8,8a octahydro-l-hydrox-5,5-dimethyl-2-methyiene-1-((E)-5 methylhex-3-en-2-yl) naphthalen-4-yl acetate. Additionally, two compounds coded as **LO-2** and **LO-3** were isolated from the chloroform extract. However because of their low amount it was impossible to characterize their structures. The sturcure of the compound was characterized using spectroscopic techniques such as IR, ^{1}H NMR,^{13}C NMR, DEPT and UV spectral data.

1. Introduction

Medicinal plants have been the mainstay of traditional herbal medicine amongst dwellers worldwide since antiquate to date. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadiar civilizations in about the third millennium BC. Hippocreates, one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, eg Chinese, Ayuvedic and Egyptian. Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs (Sarker and Nahar, 2007). About 3.4 billion people in the developing world depend on plant based traditional medicines. This represents about 80 percent of the world's inhabitants, who rely mainly on traditional medicinal plant for their primary health care (Sarker and Nahar, 2007).

According to the world health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo- pharmaceutical semi-synthesis. Such a plant will have its part including leaves, roots, rhizomes, stems, barks, flowers, grains or seeds, employed in the control or treatment of a disease condition and referred to as phytochemicals (Abo et al., 1991; Liu, 2004; Nweze et al., 2004; Doughari et al., 2009). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mbabebie et al., 2005). These compounds are synthesized by secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used on the human therapy, veterinary; agriculture scientific research and countless other areas (Vasu et al., 2009).

Plants have been used as source of medicine in Ethiopia from time immemorial to treat different ailments. Due to long history traditional medicine has in fact become an integrated part of the culture (Pankhurst, 1965). Dawit Abebe and Ahedu Ayehu reported that 80% of the Ethiopian population depends on traditional medical preparations that are of plant origin. Up until 1996, the drug research department had collected and documented over 600 medicinal plants (Abebe et al., 1996). Since the study contribute for implementing in-situ conservation, promotion and usage of the plant in a sustainable manner, and also open up the way for future research and development of new drugs from the medicinal plants. It also contributes in improving health care in the rural areas.

*Leonotis ocymifolia*is cultivated in Ethiopia for medicinal uses (Ryding et al., 2006) which include acting as ascaricide, anti cancer drug, and as a treatment against malaria, leishmaniasis, ulcers and wound (Abate et al., 1989; Habtmariam et al ., 1994). I am inerested to study on this plant, because in my local area, people use the plant as traditional medicinal to heal different diseases like fever, headaches, intestinal parasites, tape worm and wound. The other reason why, I decided to study this plant, it is abundantly available in my local area. Previously, Yeshitila Asteraye (July 2006), had studied on *L.ocymifolia* plant leaves.

1.1 Justification of the study

In Ethiopia various plants are commonly used for the disease treatment by traditional healers and community people. An estimated 80% of the Ethiopian population relies on traditional medicine. Socio-cultural appeal, accessibility, affordability, and effectiveness against a number of health problems seem to promote its widespread use. The acceptance of traditional medicine as an alternative form of health care and the development of bacterial resistance to the available synthetic drug have led to investigate active antibacterial drug from natural products. From the literature review no detailed investigation on chemical constituents of this plant. In order to fill the mentioned gap the current study designed for isolation and characterization of this medicinal plant.

1.2 Objectives of the study

1.2.1 General objective

The main objective of this study is to isolate and characterize compounds from leaves of *Leonotis ocymifolia.*

1.2.2 Specific objectives

- To extract the leaves of *Leonotis ocymifolia* using different solvent such as petroleum ether,chloroform, chloroform/methanol (1:1) and methanol successively.
- To conduct phytochemical screening tests to identify the presence of different compounds in the plant leaves.
- To isolate constituent of the plant using chromatography technique (PTLC, CC).
- To elucidate the structures of compounds isolated using different spectroscopic methods (IR, NMR and UV – VIS).

2. Literature Review

2.1 Genus *Leonotis*

The genus *Leonotis* consists of about 30 species of plants in the family Lamiaceae, vernacular (region name) Afrikaans, Lion's ear (English) (Warsson et al., 1985). One of these species is *L.ocymifolia.* It is locally known as "yeferes-zeng"(Amharic) in Ethiopia.

2.2 Geographical distribution

L.ocymifolia was wide spread in south – eastern and eastern Africa, found on rocky outcrops and in well drained soils on hill side at attitude of 1000- 2000 m (Warssan et al., 1985). The other species *Leanotis neptifolia* is native to tropical Africa and southern India (http; en Wikipedia et al).

Figure-1 Picture of the plant taken by the author.

2.3 Medicinal application of *Leonotis* species

This species is used more or less interchangeably with *L. learnues* in some areas, but its specific uses include the treatment of diabetes, hypertension, anemia, eczema and skin irritations. It is also utilized as a purgative and emmenagogue (Iwarsson et al.; 1985). In addition *Leonotis* has been used to treat various alignments including epilepsy, bits and settings, muscular cramps, respiratory disorders, headaches, viral hepatitis, intestinal warms and obesity (Iwarsson et al.; 1985).

In 1982 *L. leanurus* was shown to reduce breast tumors in mice (http// en. Wikipedia.Org et al). Its leaves are used in the treatment of hook worm (Eagle et al., 2005), intestinal parasites (Faranswonth et al., 1975).

2.4 Chemical composition of *L. ocymifolia*

Chemical tests on the plant indicated the presence of alkaloids, saponins and tannins, but not cardic or anthroquinone glycosides. The alkaloids were not of the indole or tropane type little is known about the secondary metabolites of this species of Ethiopian origin. Previous study indicated the presence of following labdane type diterpene lactones (Fig. 2) (Habtemariam et al., 1994) in the plant.

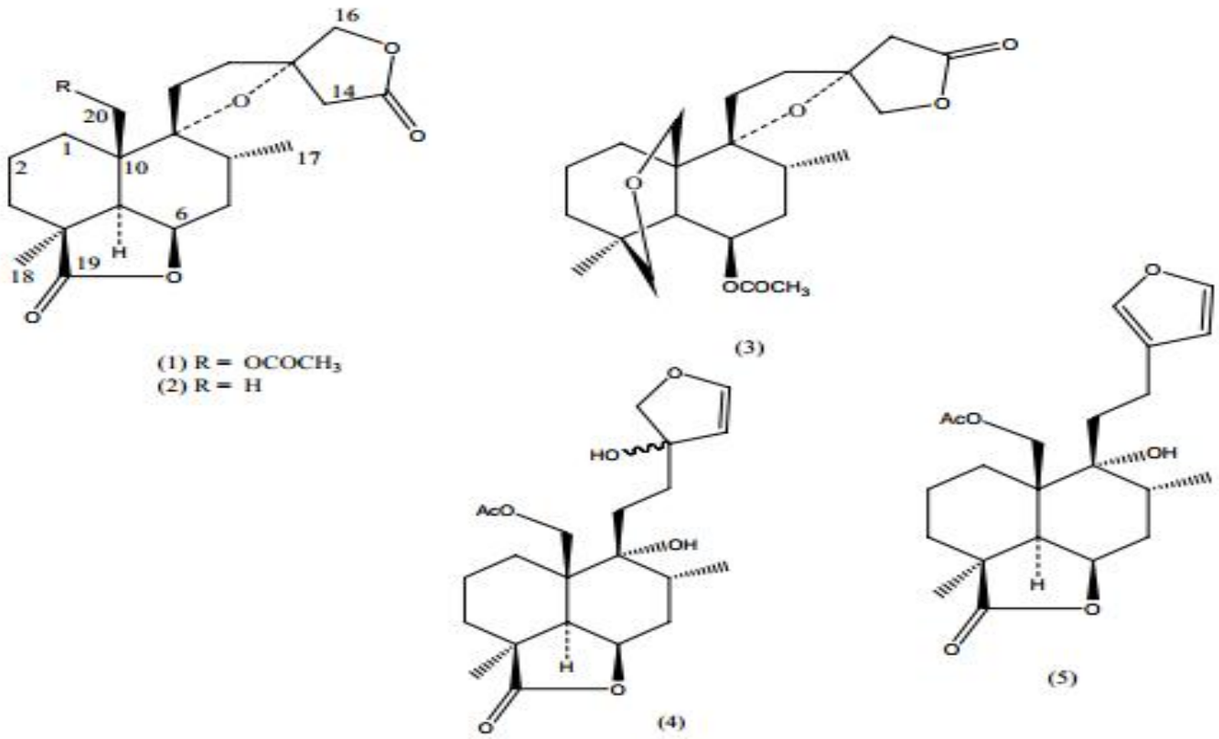


Figure 2 Diterpene lactone reported from leaves of *L. ocymifoli.*

Morrubiin is widely known diterpenoid lactones that constitute the bitter principle of the horehound and many other medicinal plants of the family *Lamiceae*. It is one of the main constituents of *Leonotis lenoures* and *Leonotis ocymifolia* (Abbonadaza et al.; 1964), which are used in several countries to treat different pathogens (fig.3) (Marrelli et al.; 2013).

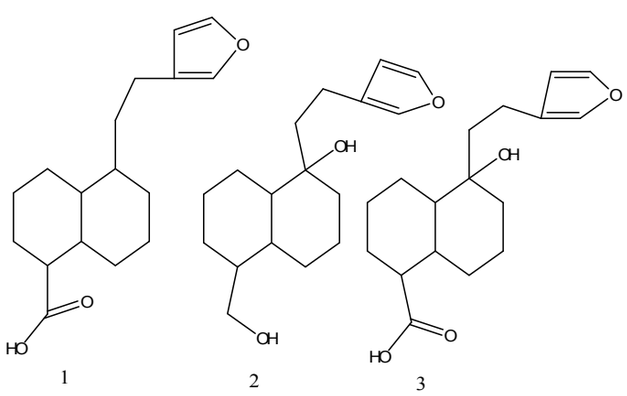


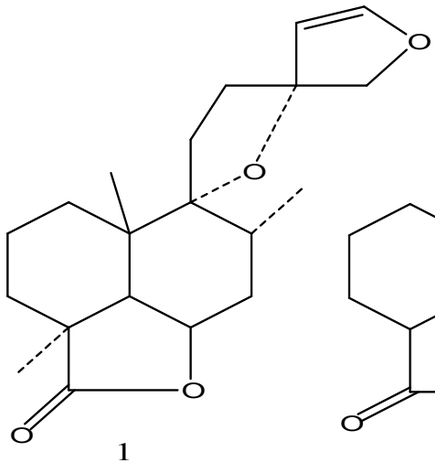
Figure-3 Different derivates of morrubiin

Leonotis lenourus has previously yielded type diterpenoid: leonurun (Kenzie et al.; 2006). New labden type diterpenoids 9, 1-3 Epoxy-6-hydroxy-labdne-16, 1-5olide and 9, 13, 15,16 Diepox-6,16 labdne diol were isolated (Habtamariam et al; 1994).

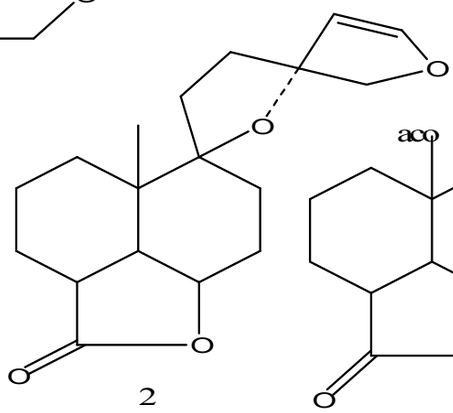
The phytochemical studies on *L.leonorus* have reported the isolation and characterization of labdane diterpenes, acyclic diterpenes, iridoid glycosides, alkaloids, dicarboxylic acids and flavonoids. In all 37 metabolites have been identified, which includes 21 labdene diterpenes. The labdane diterpenes presence in *Leonotis leonurus* and other species are chemotaxonomic marker for the genus and the mint family, *Lamiaceae* (Naidoo et al., 2011). Compounds 4,18-20 were not given names and because of their structural similarities to the other labdane diterpenes, they are here named leonleorin K(4), leonleorin(18), leonleorinM(19), leonleorinN (20) (Naidoo et al., 2011). Compounds 14-16 were also identifed as leonurenones A-C from the leaves of water extracted (fig.4) (He F et al., 2012).

Table-1 The secondary metabolites identified from *Leonotis* plants

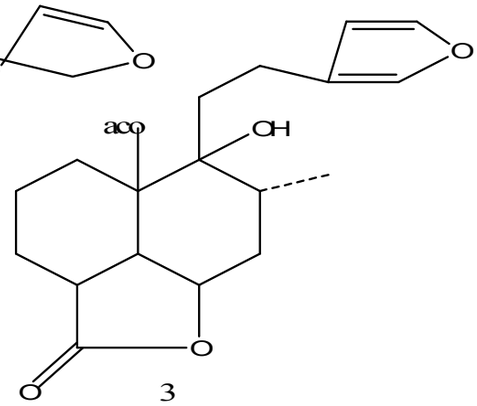
Part and extract	Compounds	Compound no	Reference
Leaves Acetone	13R-permarrubin 13S-Permarrubin Lonurun Marrubin Hispanolone	1 2 3 4 7	(Laonigro et al., 1979; Rivett et al., 1964; Kaplan et al., 1968)
Leaves Methanol, Methanol/dichloromethane	Leoleorin B/Anhydro Leoleorin C Leoleorin D Leoleorin E Leoleorin F	8, 5 9 10 11 12	(Mnonopi et al., 2011; WUH et al., 2011)
Leaves Water and Methanol	Leonurine		(Rivett et al., 1964)



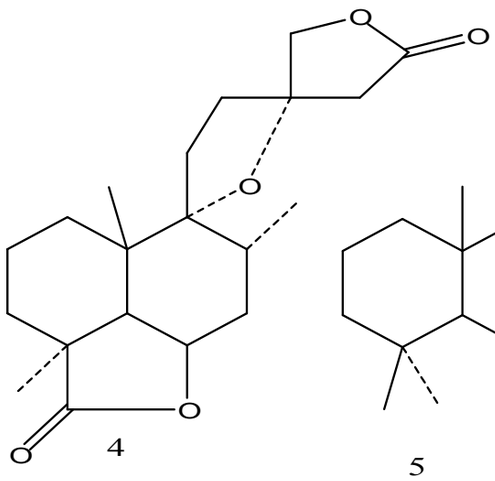
1



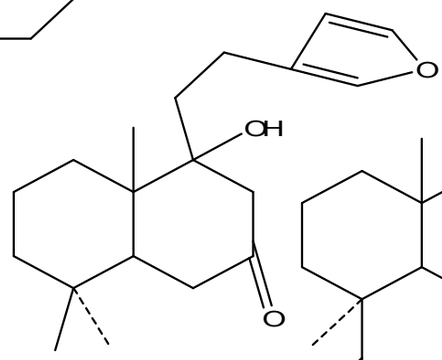
2



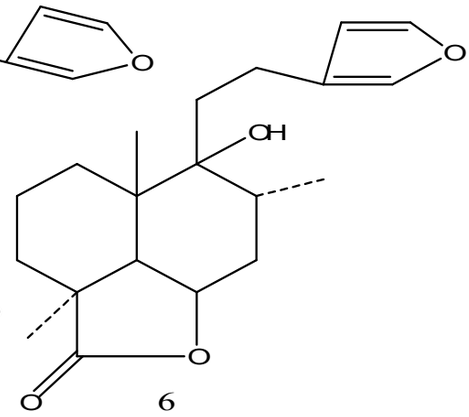
3



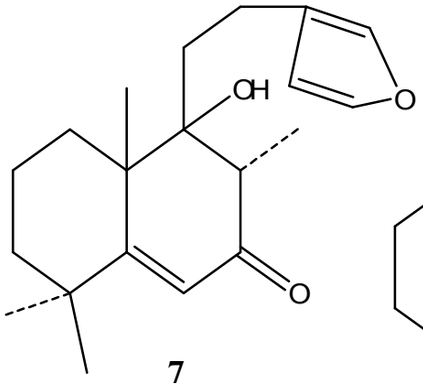
4



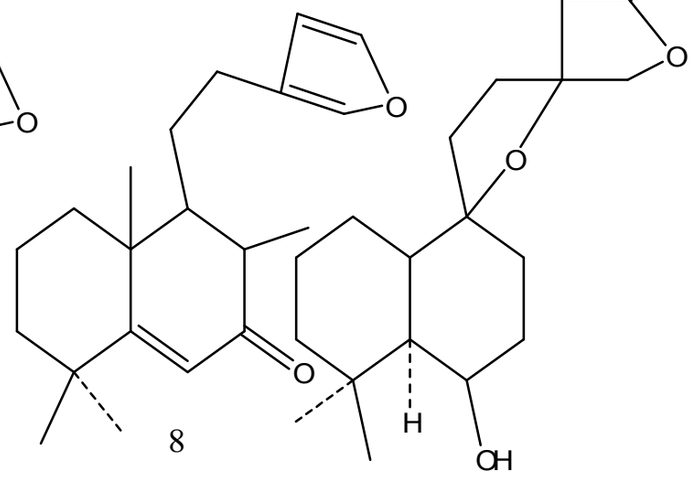
5



6



7



8

9

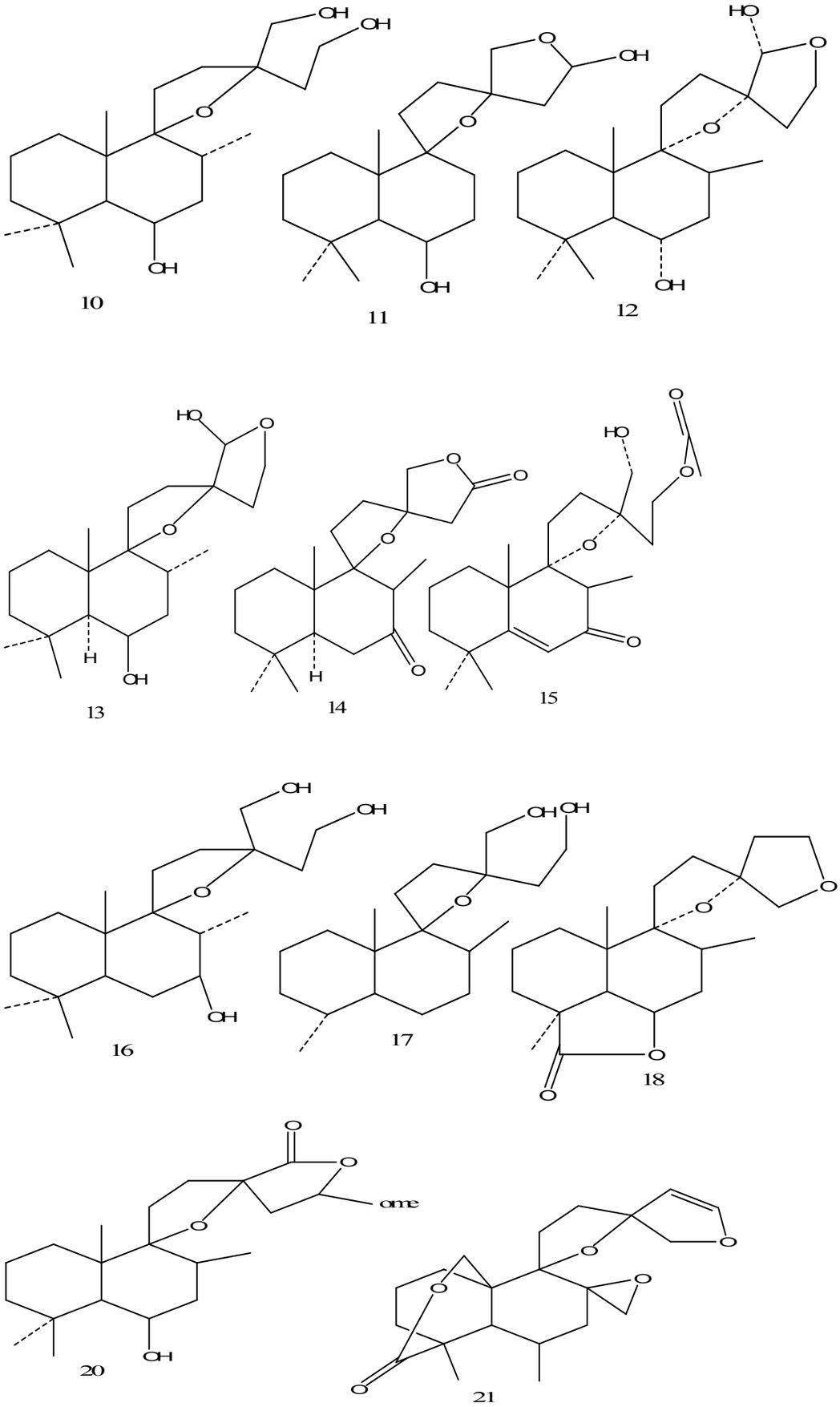


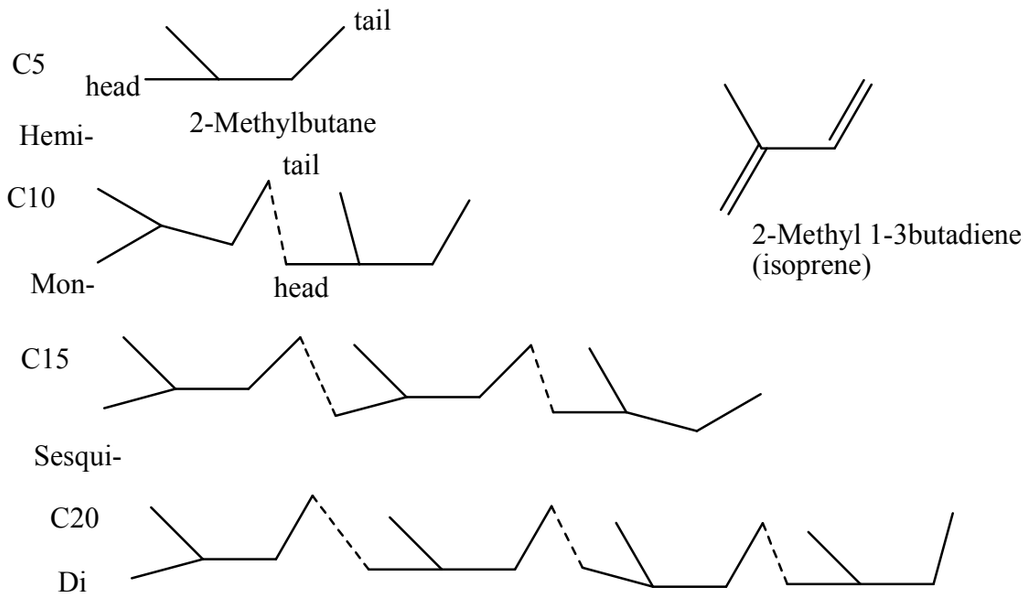
Figure-4 the secondary metabolites compounds from *Leonotis* plants

3. Terpenes

The term terpene originates from turpentine (Lat.balsamum terebinthine). Turpentine the so called “resin of up on pine trees” is the viscous pleasantly smelling balsam which flows upon cutting or carving the bark and the new wood of several pine tree species. Turpentine contains the “resin acids” and some hydrocarbons, which were originally, referred to as terpenes. The biological and ecochemical functions of terpenes have not yet been fully investigated. Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals. Less volatile but strongly tasting or toxic terpenes also protect some plants from being eaten by animals. Terpenes also play an important role as signal compounds and growth regulators of plants as shown by investigation. Many insects metabolize terpenes they have received with their plant food to growth hormones and pheromones (htt://media.wiely.com).

3.1 General structure

About 30,000 terpenes are known at present in the literature. Their basic structure follows a general principle: 2-Methyl butane residues, usually also referred to as isoprene units $(C_5)n$, build up the carbon Skelton of terpenes. In nature, terpenes occur predominantly as hydrocarbons, alcohols and their glycosides, ether, aldehyde, ketones and carboxylic acids and esters (scheme.1) (htt://media.wiley.com et al).

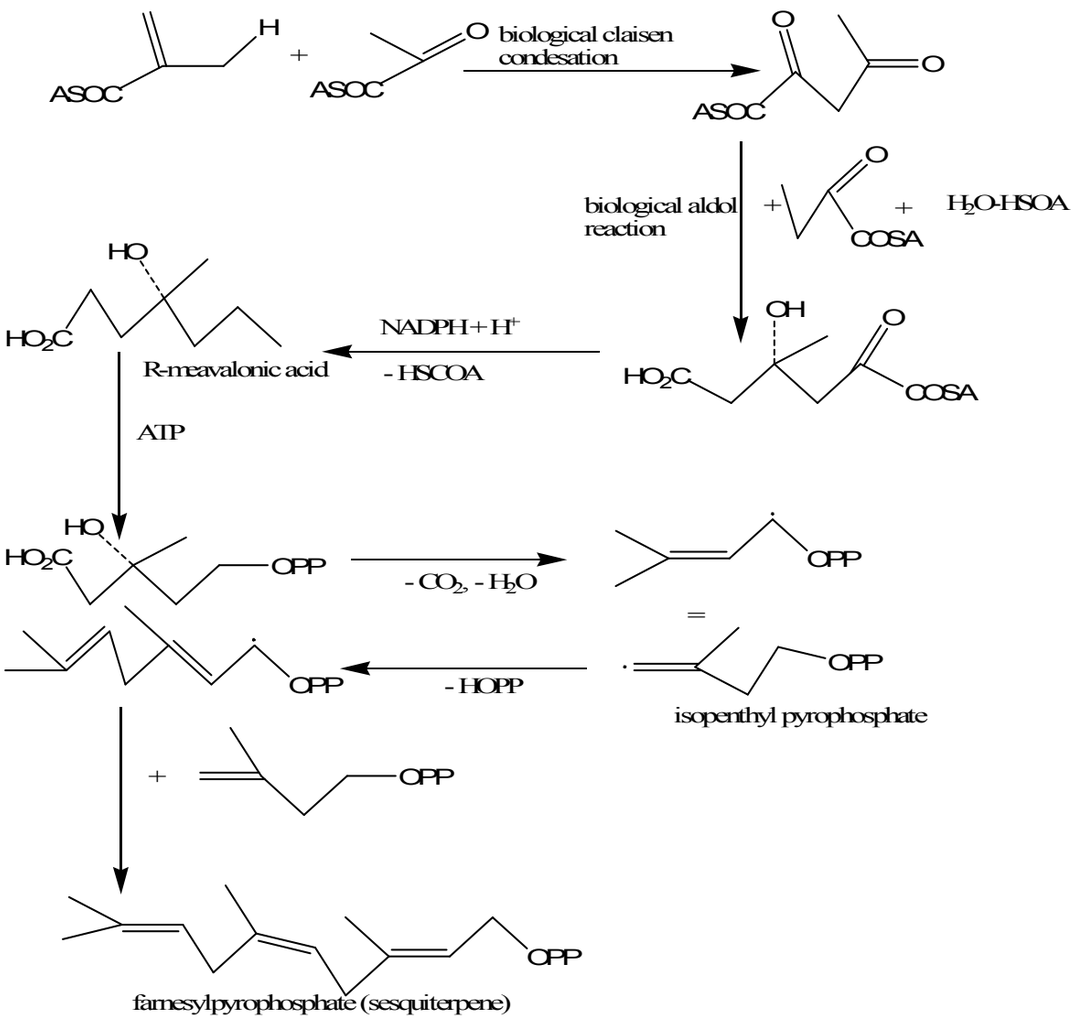


Scheme-1 Parent hydrocarbons of terpenes (isoprenoids)

3.2 Biosynthesis

3.2.1 Sesquiterpenes

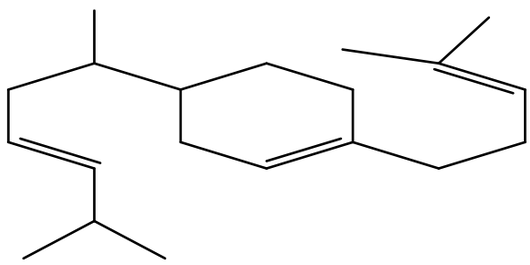
A large structurally varied and botanically closely allied class of sesquiterpenes is group of terpenes found distributed through plants of the family composite (scheme.2) (Sewram et al; 2000).



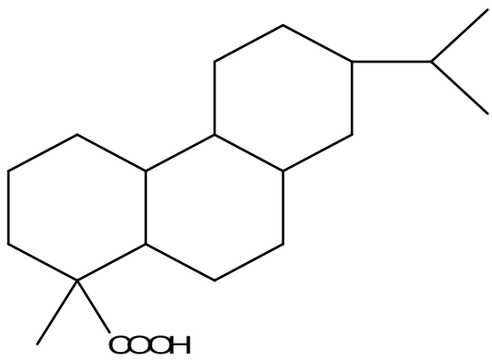
Scheme 2 Biogenesis of sesquiterpene

3.2.2 Diterpenes

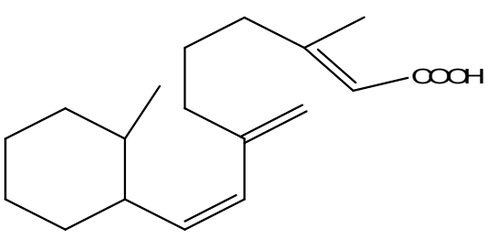
The diterpenes are a widely varied group compounds based on four isoprene groups, most of which are of limited distribution in the plant kingdom. Because of their higher boiling points, they are not considered to be essential oils, instead they are classically considered to be resins, the material that remains after steam distillation of aplant extract. The diterpenes exist in a variety of structural types (show in scheme-3) (Bornemann et al, 1988).



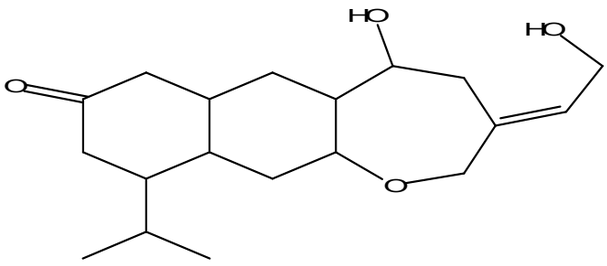
x-camphorene



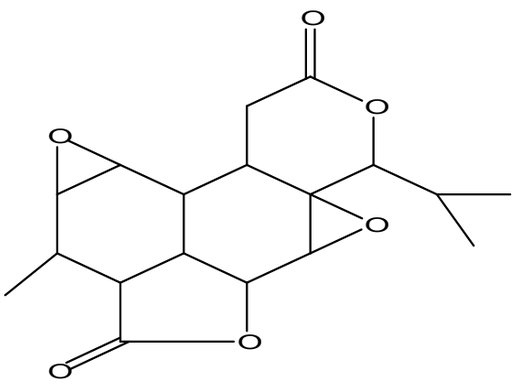
Abietic acid



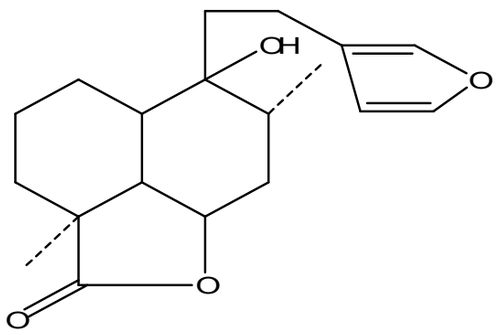
Phyto



Zoopatonol



Inumakilacetone

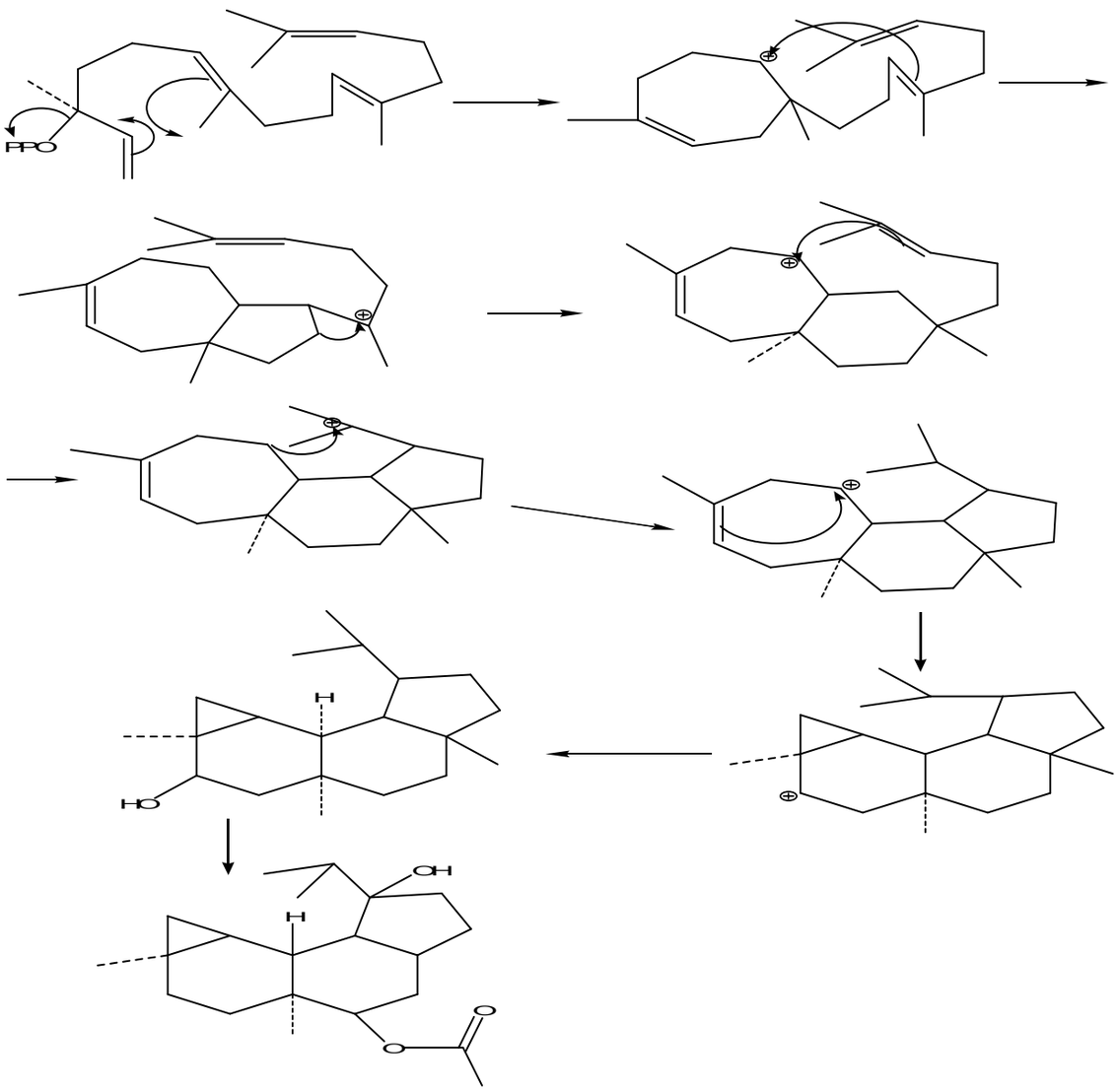


Marrubin

Scheme-2 some common plant diterpenes

Many interesting examples may be mentioned. The cyclic ether zapotonol is drived from the Mexican plant, montanoa tomentose and has been used as an abortificient. A verity of cythotoxic lactone have been isolated from podocarpus species (Bornemann et al, 1988). Marrubin is a diterpenes lactone from white horehound (murrubium vulgare), which has been used as a bitter and choleretic in digestive and billitary complaints (Bornemann et al., 1988).

The diterpene verrucosan-2β-ol from the green phytotrophic eubactrium chloroflexus aurantiacus in biosynthesized viamevalonate. The cyclization process is initiated by solvolysis of gerany linalyl diphosphate leading to a bicyclic intermediate, which undergoes as 1, 2 rearrangements, to generate a tricyclic system.



Scheme-4 biosynthesis of diterpene

4. Materials and Methods

4.1. Plant material

The leaves of *Leonotis ocymifolia* were collected from Sire Woreda, Oromia reign, Arsi zone, Ethiopia in December 2014 about 148 km far from Addis Ababa. The plant specimen was identified and deposited at National Herbarium, Department of Biology, in Addis Ababa University.

4.2. Chemicals and instruments

Mortar and Pestle, Rota evaporator, Whatman No.1 filter paper, graduated cylinder, beakers, test tubes, pipettes, TLC Plate (pre-Coated aluminum sheet silica gel 60 F254), shaker, column, Silica gel (70-230Mesh).

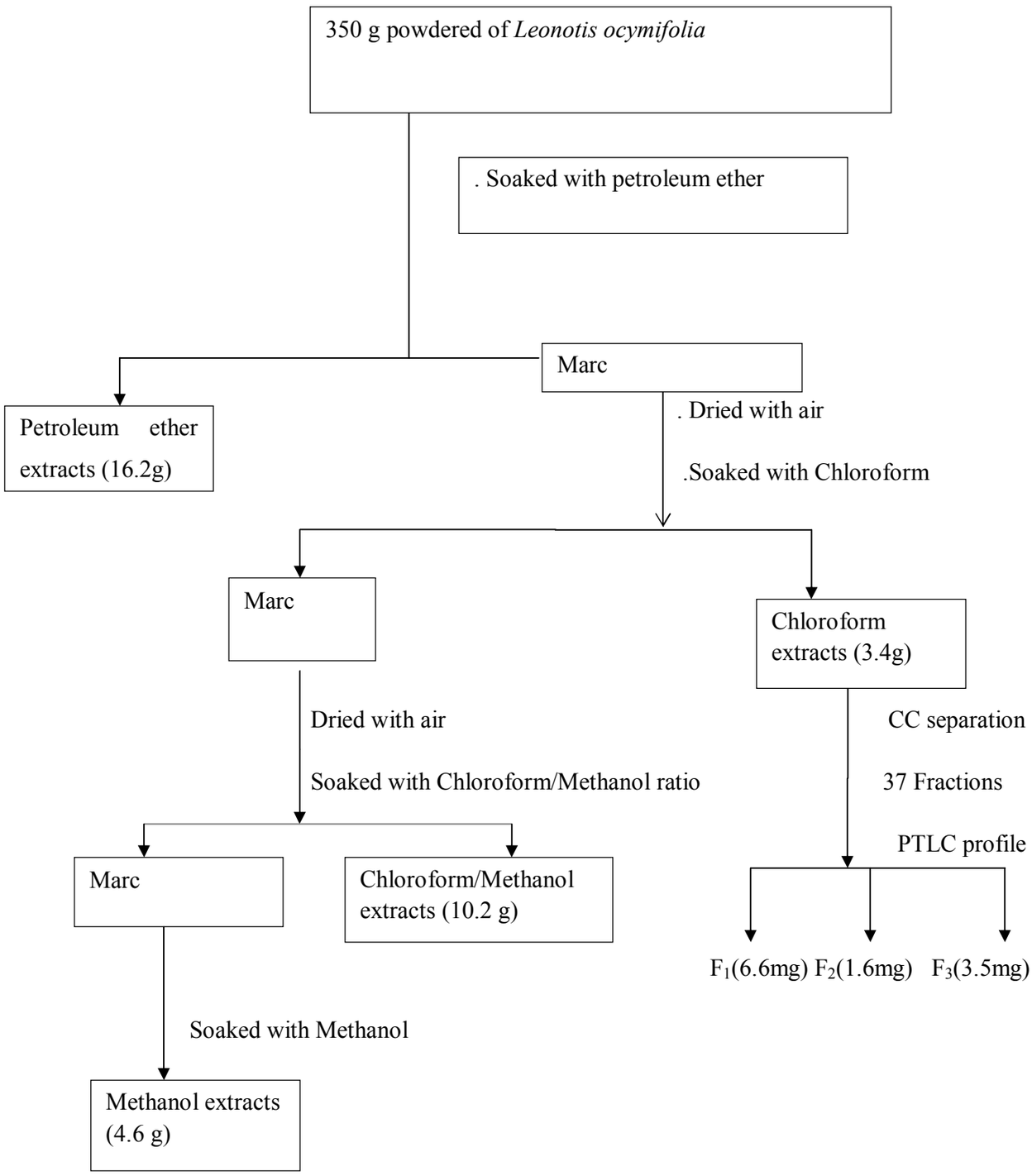
IR spectrum was recorded as KBr pellets.

^{1}HNMR, ^{13}CNMR spectra was recorded on a Bruker advance 400MHz spectrometer with TMS as internal standard.

Analytical TLC was done on 0.2 mm thick layer silica gel on aluminum card and detection was done using varillin reagent for terpenoids. Column chromatography was carried using silica gel 60 (Merck).

4.3 Extraction

The powder of air dried leaves of *Leonotis ocymifolia* (350 g) was extracted with different solvents successively and yielded petroleum ether extract (16.2 g), Chloroform extract (3.4 g), Chloroform/Methanol (1:1) extract (10.2 g) and methanol extract (4.6 g) at room temperature for 3 days each. The solvents were evaporated using rota vapor.



Scheme.5 Extraction Outlines

4.4 Coding system

In the coding system **L** stands for the genus name *Leonotis*, **O** stands for species name *ocymifolia*, the number attached to LO indicate the position of the compound starting from the highest Rf value to the lowest. Thus, **LO-1** stands for the first compound.

4.5 Thin layer chromatography Analysis

TLC is often used to determine the best solvent system for column chromatography. Each crude extract was analyzed using TLC to select best solvent system for CC. The crude extract with good TLC profile was subjected to CC and eluted with solvent system of increasing polarity.

4.6 Isolation of chloroform extracts using CC

The solvent free Marc was then soaked with chloroform for 72 hours to give chloroform crude extract (3.4 g) and analyzed using TLC. After column chromatography was packed with silica gel. The column was eluted with the following solvent system and 37 fractions each 25mL were collected (Table.3).

Chloroform extract	Solvent system	Ratio	Volume(ml)	Fractions	Combined fractions
	Petroleum ether		200	F_1-F_6	F_4,F_5,F_6
	Petroleum ether/ethyl acetate	9:1	100	F_7-F_9	
	Petroleum ether/ethyl acetate	8:2	100	F_{10}-F_{13}	F_{12},F_{13}
	Petroleum ether/ethyl acetate	7:3	100	F_{14}-F_{17}	
	Petroleum ether/ethyl acetate	6:4	100	F_{18}-F_{20}	F_{20},F_{21}
	Petroleum ether/ethyl acetate	5:5	100	F_{21}-F_{22}	
	Chloroform		200	F_{23}-F_{24}	
	Chloroform/methanol	9:1	100	F_{25}-F_{27}	
	Chloroform/methanol	8:2	100	F_{28}-F_{29}	F_{28},F_{29}
	Chloroform/methanol	7:3	100	F_{30}	
	Chloroform/methanol	6:4	100	F_{31}-F_{33}	
	Chloroform/methanol	5:5	100	F_{34}-F_{35}	F_{34},F_{35}
	Methanol		100	F_{36}-F_{37}	F_{36},F_{37}

Out of 37 fractions only fraction 18 showed three spot having pale yellow, blue black and yellow color.

Fraction18 was analyzed using TLC to select best solvent system for PTLC and the best solvent system was n-hexane/ ethyl acetate (4:1). Fraction 18 was further purified using PTLC (solvent n-hexane/ethyl acetate system).

Table-4 The PTLC profile purified fractions obtained from F_{18}.

Fraction	Code	Amount (mg)
F_1	LO-1	6.6
F_2	LO-2	1.6
F_3	LO-3	3.5

4.7 Phytochemical tests

4.7.1 Detection of alkaloids

Extracts were dissolved in dilute Hydrochloric acid and filtered. Then Filtrates were treated with Wangner's reagent (iodine in potassium iodide). Formation of reddish precipitate indicated the presence of alkaloids.

4.7.2 Detection of saponins

About 0.5g of each plant extract was shacked with 2 ml of water in a test tube. Foam does not produced afeter ten minutes which indicated the absence of saponins.

4.7.3 Detection of tannins

A bout 0.5g of plant extract was stirred with 10 ml distilled water, filtered, and ferric chloride reagent was added to the filtrate .A blue-black color precipitate was observed as evidence for the presence of tannins.

4.7.4 Detection of terpenoids

About 0.5 g of plant extract was dissolved in 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added to it and heated about for about 2 minutes. A grayish color was observed as evidence for the presence of terponoids.

Table-5 Phytochemical test

Test	Extract Chloroform
Alkaloids	+
Saponins	-
Taninns	+
Terpenoids	+

+ Present - Absent

5. Results and discussion

5.1 Characterization of compound LO-1

The compound LO-1 was isolated as a pale yellowish crystal with Rf value of 0.45 (petroleum/ethyl acetate; 9:1). It gave a pale yellow color spot on TLC when viewed under UV light of 366 nm. The UV spectrum (appendix-1) displayed an absorption bond at λ max 214 nm indicated the presence of olefinic system.

In the IR (kBr) spectrum (appendix- 2) the absorption band at 3435 cm^{-1} showed the OH stretching that indicates the presence of hydroxyl group. The strong absorption bond at 2923 cm^{-1} showed the presence of the C-H stretching for methyl groups. The absorption bond at 1464 cm^{-1} showed the presence of the olefinic C=C stretching. The absorption bond at 1728 cm^{-1} showed the presence of ester group.

The ^{1}H NMR spectrum (Appendix-3) of LO-1 in $CDCl_3$ suggested the presence of six peaks at δ 0.96, 0.96, 1.30, 1.30, 1.96 and 2.15 indicate the methyl groups. The peaks from δ 0.96 and 1.00, 2.03 and 2.06, 1.96 and 2.15, 2.51, 4.98 and 5.03 indicate five methylene groups. The peaks at δ 2.26, 2.33, 2.37, 5.82 and 5.84 indicate five methine groups.

The ^{13}C NMR spectrum (appendix-4) analyzed with the aids of DEPT-135 (appendix-5), showed six quaternary carbons, which were attributable to three olefinic, three oxygenated carbons. In addition, five methine, five methylene, six methyl and two symmetric carbon attached to quaternary carbon and two symmetric carbon attached to methine group were observed. One quaternary carbon attached with hydroxyl group and one methyl attached carboxyl group.

Table-6 Proton Decoupled ^{13}C NMR and DEPT-135 spectral data of compound **LO-1**

No Carbon	^{13}C NMR (ppm)	DEPT(ppm)	Remark
1	29.17	down	CH_2
2	14.11	down	CH_2
3	31.44	down	CH_2
4	25.72	-	C(quaternary)
5	119.10	-	C(quaternary)
6	148.67	-	C(quaternary)
7	29.52	down	CH_2
8	134.80	-	C(quaternary)
9	86.6	-	C(quaternary)
10	30.14	up	CH
11	33.83	up	CH
12	125.20	up	CH
13	139.27	up	CH
14	31.96	up	CH
15	29.70	up	CH_3
16	171	-	C(quaternary)
17	28.96	up	CH_3
18	29.36	up	CH_3
19	114.06	down	CH_2
20	22.17	up	CH_3

Table-7 Proton NMR spectral data of **LO-1**

No Carbon	Proton no	^{1}H NMR(ppm) and (J)
1	H-1	1.0 and 0.96, m
2	H-2	2.03 and 2.06, dd (J_1=4HZ, J_2 =12HZ)
3	H-3	2.15 and 1.96, s
7	H-7	2.51, s
9	OH	2.15, s
10	H-10	2.26, d (J=12Hz)
11	H-11	2.37, t (J=8HZ)
12,13	H-12,13	5.82 and 5.84, m
14	H-14	2.33, d (J=8 Hz)
15	H-15	0.96, m
17	H-17	1.96, s
18	H-18	1.30, m
19	H-19	4.96 and 5.02, dd (J_1=8HZ, J_2 =8HZ)
20	H-20	0.96 , d (J= 4Hz)

From comparison of the ^{13}C NMR spectral data of **L.O-1** with literature, compound **LO-1** closely resembles morrubin compounds structure (Naidoo et al,. 2010; HeF et al., 2012)(Figure-4).

From all the above data the following structure was suggested for compound **LO-1**

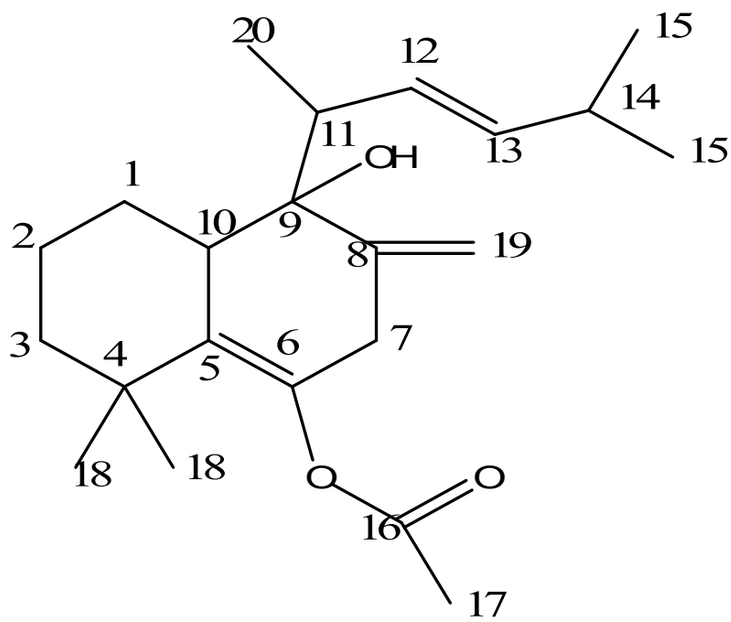


Figure-5 Proposed structure of **LO-1**

6. Conclusions and Recommendations

The leaves of *Leonotis ocmyifolia* is used as a traditional medicine for treatment of intestinal parasites, headache, skin irritation in east Oromia. The plant leaves was extracted with petroleum ether, Chloroform, $CHCl_3$/MeOH (1:1) and MeOH successively. Based on TLC analysis the $CHCl_3$ extract was selected for further purification.

The chloroform extract of leaves of the plant after fractionation with column chromatography and further purification with PTLC gave the compound coded as **LO-1** belonging to diterpene class of compound namely 1,2,3,5,6,7,8,8a octahydro-1-hydrox-5,5-dimethyl-2-methylene-1-((E)-5 methylhex-3-en-2-yl) naphthalen-4-yl acetate.

Further investigation to elucidate the complete and correct structure of **LO-1** additional (2D NMR, HRMS) experimental data are recommended.

Based on TLC analysis anyone who would like to continue his/her study on this plant better to do on chloroform extract, since these extract have more compounds in it.

Thus, further tests are needed to evaluate activities of the compound against different bacterial species to explore all possibilities to evaluate potential of the isolated compounds as lead in the development of antibacterial agents. Hence, much more phytochemical and biological study should be carried out on the plant in future.

References

Abebe,D., Proceedings of the work shop on Development utilization of herbal remedies in Ethiopia (preface). Diterpenes from the leaves of *Leonotis ocymifolia var. raineaiana* Journal of Natural product 1996: 57(1): 1570 – 1574.

Abebe,D. and Ayehu,A., Medicinal plant and enigmatic heath practices of northern Ethoipia. B.S.P. E, Addis Ababa, Ethiopia 1993.

Abo,k.A., Ogunleye,V.O. and Ashidi,J.S., Antimicrobial potential of spandiasmambin, cotonzambesicus and zygotritoniacrocea. Journal of pharmacological research 1995: 5(13): 494 – 497.

Barano(x) ska,I., H.Barchanska and E.pacak, procedures of trophic chain samples preparation for determination of triazines by HPLC and metals by ICP – AES methods, Environmental pollution 2006, 143, 206 – 2 GHP 2007. Ghana Herbal pharmacopeia council for scientific and industrial research (CSIR) ACCRA, 295pp.

Bornemann, N., Patteerson, G.M.L. and Moore, R.E., J.Am.Chem.Soc, 1988, 110, 2339.

Brachet,A., Rudaz,S., Mateus,L., Christen,P. and Veuthey,J., optimization of Accelerated solvent Extraction of cocain and Benzoylecgorine from coca leaves, J. sep. sci. 2001, 24, 865-873

Coddl: Lamiaceae: flora of southern Africa,south Africa 1985, 28 (4), p.32.

Dask, Tiwari,R.K.S., Shrivastava,D.K., Techniques for evaluation of medicinal plant products as antimicrobial agent: Current Methods and future trends. Journal of medicinal plant Research 2010; 4(2): 104-111.

Doughari,J.H., and Obidah,J.S., (2008). Antibacterial potentials of stem bark extracts of *Leptadenia lancifolia* against some pathogenic bacteria. Pharmacology anilines 2008:3, 172 – 180.

Doughari,J.H., Human,I.S., Bennade,S. and Dakidemi,N., Antibiotic resistant verocytotox in producing bacteria. Journal of medicinal plant research 2009: 3(11) 839-848. Dluquedecastro,M., preliminary steps of the analytical process. From sampling to detection, pure Appl. Chem. 2002, 74,223 – 2298

Eagle.A; South African Journal of Botany 2005. 7 (1), 114 – 116.

Edoga,H.,OOk,w.w., D.Embaebie,B.O., phytochemicals constituents of some Nigerian medicinal plant. Afr. S. Biotechol 2005: 4(7); 685- 688

Eloff,J.N, which extracting should be used for the screening and isolation of antimicrobial components form plants. Journal of Ethno pharmacology 1998; 60:1-8Ethiopian medical Journal 3: 157 – 172.

Evans, W.C., (2002) pharmacognose of: Landon, W.R. Saunders – persions G.J. and M.W. Quim by (1976) Nigerian plant III photo chemical screening for alkoids, saponis, tannins. J. pharms ci, 56 (2), 1512.

Faransworth,M.R.; potential value of plants as sources of new anti-fertility agent. J. pharm –sic 1975, 64(4), 535 – 598.

Firn,R., Nature chemicals, Oxford University press, oxford. Pp74 – 75 Madziga HA, sinnis and sandabe UK. Phytochemical and elemental Analysis of Acalyphawilkesianaleag. Journal of American science 2010: 6(11): 510 – 514.

Gelahun,A., Etsedebdabe (Ethiopian Traditional Medicine) (in Amharic) - Addis Ababa University, Addis Ababa1998.

Harborne, J.B., Phytochemicals Method, Chapman and Hall Ltd. London, 1973:PP. 49-188.

He,F., Lindqvist,C., Harding,w.w., LeonurenonesA-C; Labdane diterpenes from *Leonotis leonurus*. Phytochemistry 2012; 83:168-172.

Habtemariam,S., Gray,A.I., and water man p.G (1994).Diterpenes from the leaves of *Lonotisocymifoliavar.raineaiana* Journal of Natural product 57(1): 1570 – 1574.

I warsson, M., (1985) the genus *Leanotis,* flora of southern Africa. 28(4):31 – 37.

Kaplan,E.R., Rivett, D.E.A., Structures of compounds x and y, two Labdane diterpenoids, from *Leonotis leonurus*. J chem soc *C* 1968; 265-266.

Kar,A., (2007). Pharmasegnosy and pharmacopeia technalogy (Revised – Expanded secand Edition). New age international Limited publishers New Delhi.Pp 332 – 600.

Laonigro,G., Lanzetta.R., parrilli,M., Adinolfi,M., Mangoni,L., The configuration of the diterpene spiro ethers from *morrubium vulgare* and *Leonotis lenurus*. Gazz chin Ital 1979; 109 (3/4); 145-150.

Liu, R.H., (2004).potential synergy of phytochemicals in cancer prevention, mechanism of action Journal of nutrition. 134 (12 suppl): 34795 – 34855.

Marthinez,M.J.A.; Lazaro, R.M delolmo LMBB Benito, P.b. (2008) anti – infectious activity in the anthemideae tribe. In: Atta – Ur _ (Ed.) studies in Natural products chemistry vol.35. Elsevier. pp 445.

Menasse,G., The use and value of wild plants to the people of bale walia. 1991, 18:21-28.

Mnonopi,N., Ruby-Ann.R,Davies Coleman,M.T., Frost,C.L., The cardio protective facts of morrubiin, a diterpenoid found in *leonotis leonurus* extracts. J. Ethnopharmaceol 2011: 138(1); 67-75.

Mungai,G.M., some medicinal plants of Ambo seli National park and their uses: in conversatio and utilization of indigehaus Medicinal plant and wild relatives of food crops,1997, p.53 -56.

Naidoo,D., Maharaj ,V., Crouch,N,R., Ngwane,A., New Labdane type diterpenoids from *Leonotis lenurus* support circumscription of Lamiaceae sl. Bio chem syst *Ecol* 2011: 39(3); 216-217.

Neub, N.S., Afolayon,A.J., Okoh, A. I., Assessment Techniques of Antimicrobial Properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008: 7(12): 1797-1806.

Nweze,E.L., Okafor,J.L., and Njok. (2004). Antimicrobial activates of methanolic extracts of Obute G.C – 2007. Ethno medicinal plant resources of south eastern Nigeria,African.

Pankhurst,R.,1965 An historical examination of traditional Ethiopian medicine and surger Sofowarea.A. Medicinal plants and tradional Medicine in Africa:Ibadan, spectrum book limited.

Parekh,J., Karathia,N., Chands'. Evaluation of antibacterial activity and phytochemical analysis of Bauhinia Variegata. L. Bark. African Journal of Biomedical Research 2006; 9: 53-56.

Remington,J.P., Remington: The Science and Practice of pharmacy 21st edition, Lippincott Williams and Wilkins 773-774. Cowan MM. Plan products as antimicrobial agents. Clinical Microbiology review 1999, 12(4): 564-582.

Rivett,D.E.A., Isolation of marrubiin from *Leonotis leonurus*. J chem. Soc 1964: 1857-1858.

Sarker,S.d., and Nahar,L.,(2007) Chemistry for paharmacy students General organic and natural product chemistry England Jhonwiley and sons. Pp 283 – 359.

Stephen, K.S., Medicinal plant alakoids. The university of Toronto press, Toranto,Canada 1970.

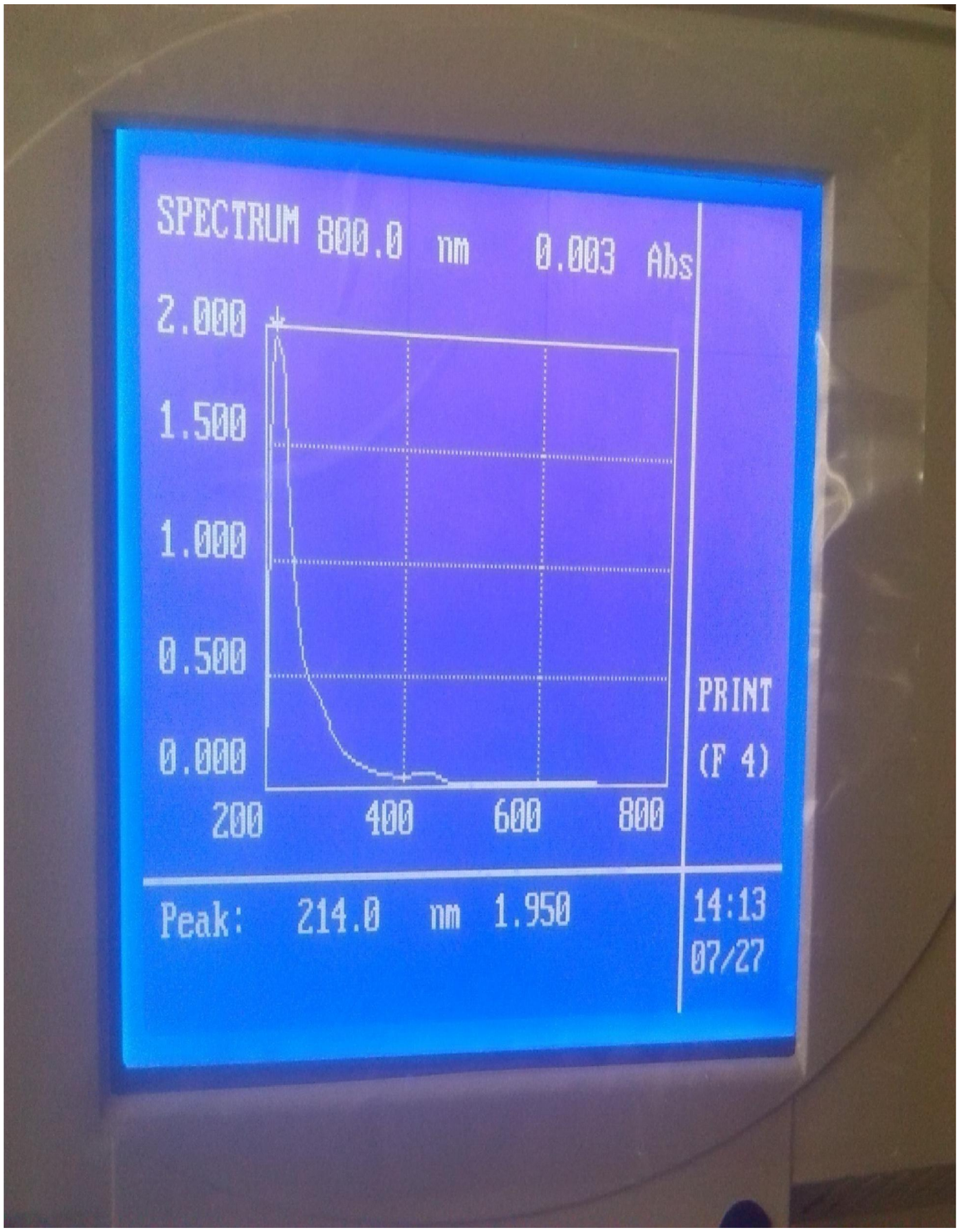
Trumeguinessis (sochumn and Thorn) and morinda Lucinda used in Nigerian Herbal Medicinal practice. Journal of Biological Research Niotechnology. 2(1): 34 – 46.

Vasu,K., Good, Suryam,S.V., Singasa,S., chary,M.A., Biomolucalar and phytochemical a nalyses of three aquatic angiospams. Afr. S. Microbiol Res., 2009:3(8): 418 – 429.

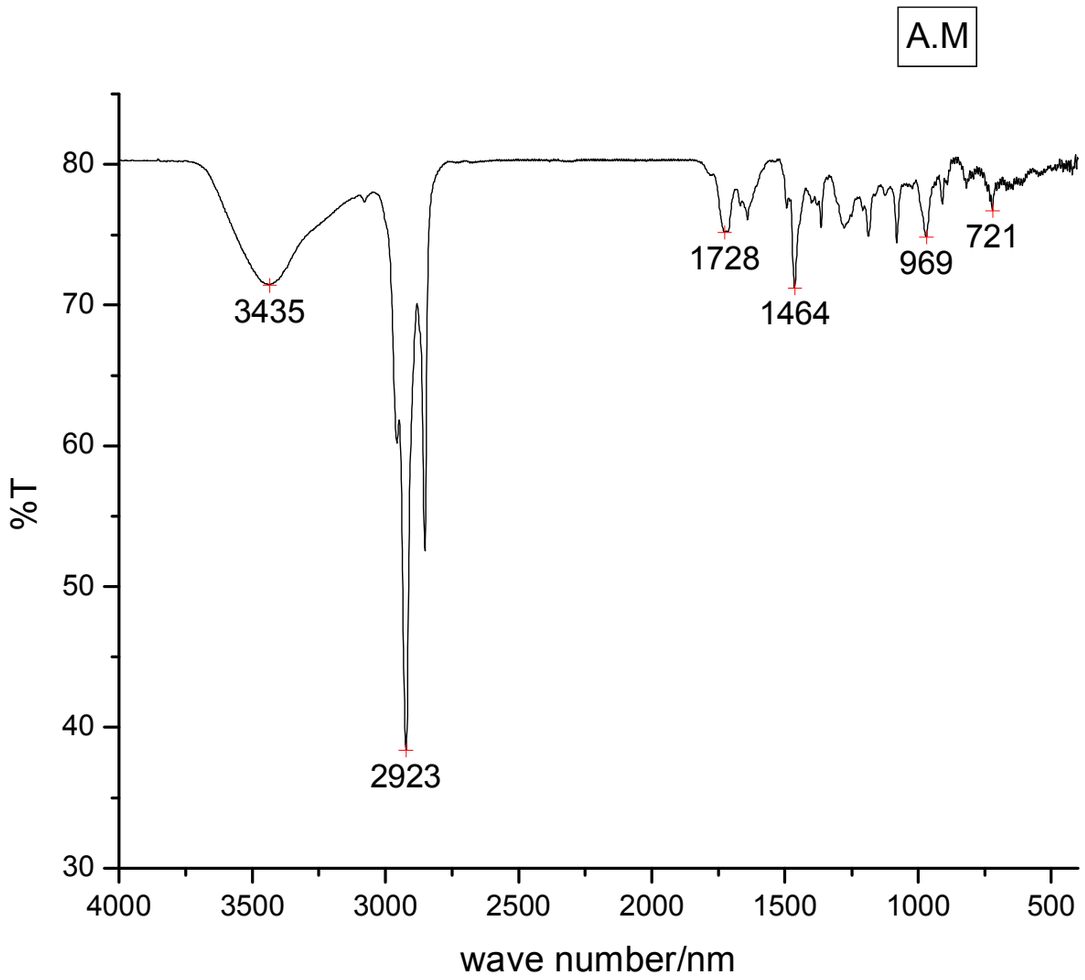
Vandenberg, H.J., Cliford, K.D., Bartle,J.,caroll and I.D. Newton, Analyst,1999,24,397- 400 S. Stipicevec, Finge,S.L.Z., krajj and Drevenker,V., comparison of Gas and High performance Liquid chromatography with selective detection for Determination of forazine Herbicides and their Degradation products Extracted ultrasonically from soil, *J.sep. sci. 2003, 26 1237 – 1246.*

Appendices

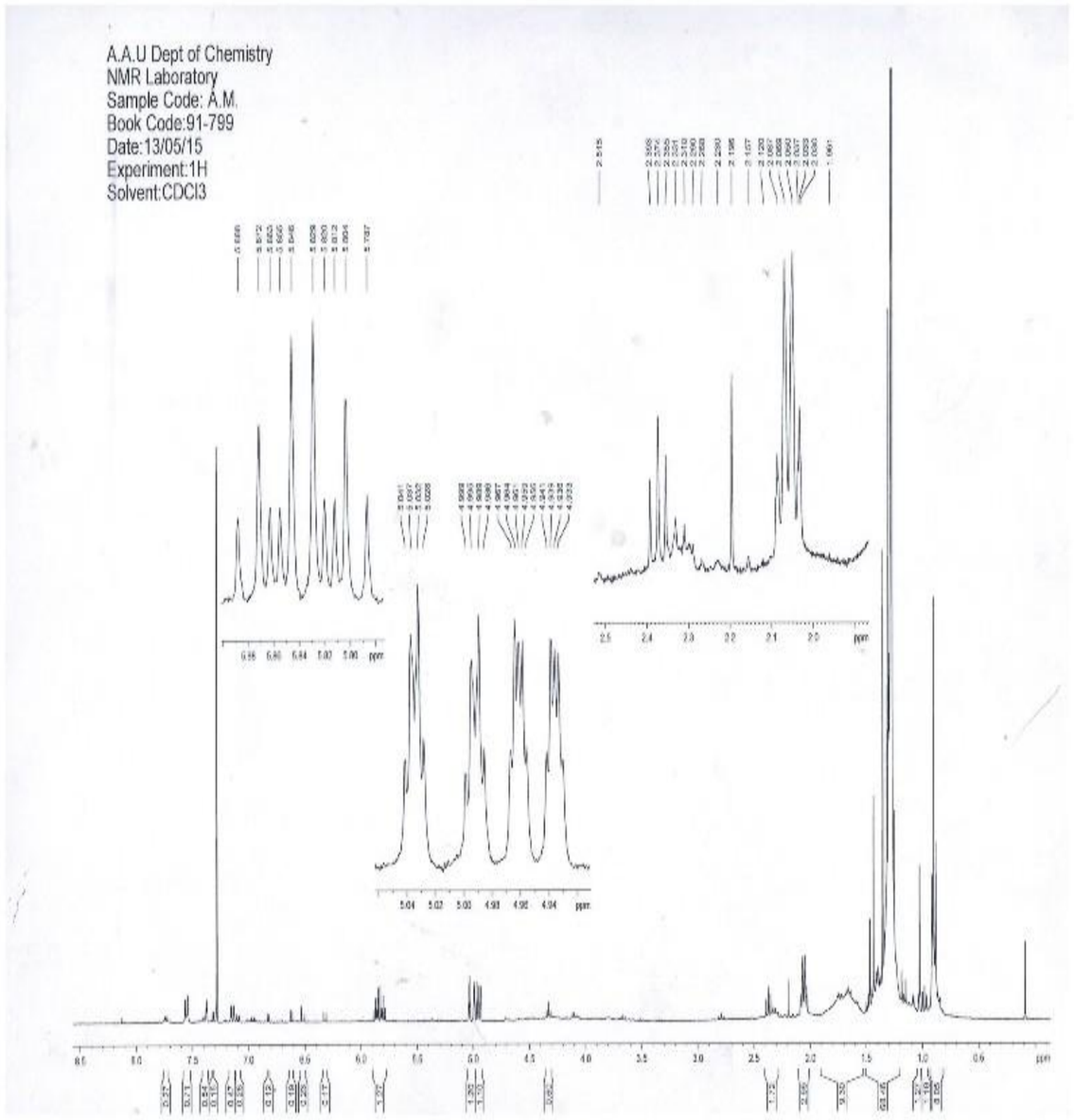
Appendix-1 UV Spectrum of **LO-1**



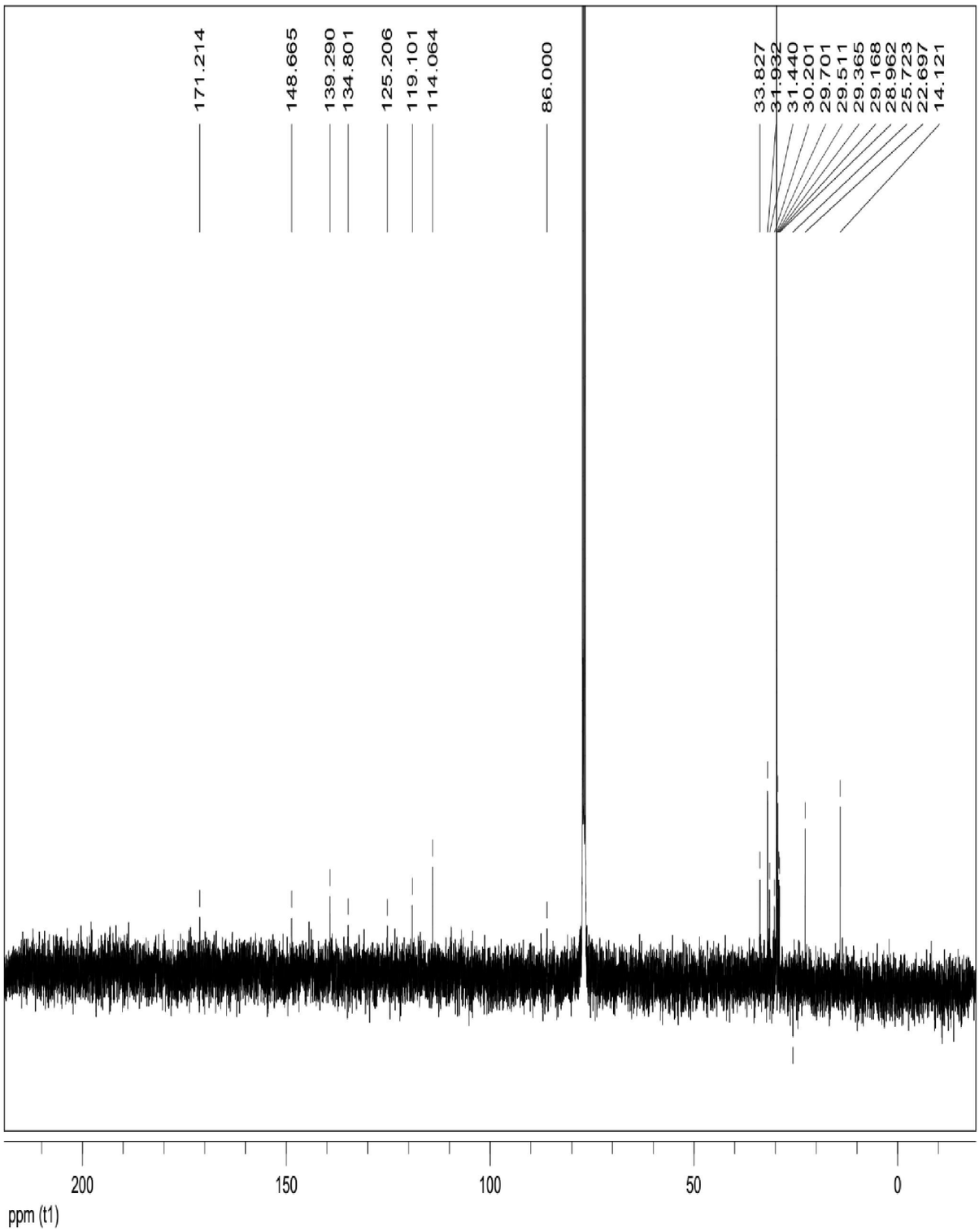
Appendix-2 IR Spectrum of **LO-1**



Appendix-3 ^{1}H NMR spectrum of **LO-1** in $CDCl_3$



Appendix-4 ^{13}C NMR spectrum of **LO-1** in $CDCl_3$



Appendix-5 DEPT-135 spectrum of **LO-1**