

**ISOLATION AND STRUCTURAL ELUCIDATION OF
COMPOUNDS FROM THE ROOT AND AERIAL PARTS OF
*Dicliptera laxata***



A Thesis Submitted to Program of Applied Chemistry

School of Applied Natural Sciences

Adama Science and Technology University

By: Araya Demissie

Advisor : Aman Dekebo(PhD)

Adama Science and Technology University, Adama

September 2016

APPROVAL SHEET

ADAMASCIENCE AND TECHNOLOGY UNIVERSITY SCHOOL OF APPLIED NATURAL SCIENCES PROGRAM OF CHEMISTRY

As Thesis Research advisors, we hereby certify that we have read and evaluated this Thesis entitled: “**Isolation and structural elucidation of compounds from the root and aerial parts of *Dicliptera laxata***” under our guidance by **Araya Demissie**.

.

We recommend that it be submitted as fulfilling the thesis requirement.

_____	_____	_____
Major Advisor	Signature	Date

_____	_____	_____
CO-Advisor	Signature	Date

As a member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read, and evaluated the Thesis prepared by **Araya Demissie** and examined the candidate. We recommended that the thesis be accepted as fulfilling the thesis requirements for the Degree of Master of Science in Chemistry.

_____	_____	_____
Chairperson	Signature	Date

_____	_____	_____
Internal Examiner	Signature	Date

_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent up on submission of its final copy to the Dean’s office of graduate studies through the candidate’s program.

STATEMENT OF THE AUTHOR

By my signature, I declare and affirm that this thesis is my own work and all source of materials used for this thesis have been dully acknowledged. This thesis has been submitted in partial fulfillment of the requirement for M.Sc. Degree in Chemistry at Adama Science and Technology University and is deposited at the University Library to be made available to borrow under rules of the Library. I gravely declared that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis are allowed without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head or the Dean School of Applied Natural Sciences or the Head of Applied Chemistry Program when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

Name of the author: Araya Demissie **Signature** _____

Place: Adama Science and Technology University, Adama

Program: Applied Chemistry

Date of Submission: September 7, 2016

DEDICATION

This work is dedicated to Mimihir Mitiku Alef (my grandfather), Captain Demissie woldetsadik (father), Seblewngel Mitiku (mother), Simegnish Teklay (wife), Bitsiatemariam Araya (daughter), Ermias Araya(son) and Feben Araya (daughter)

Acknowledgments

I would like to present unbearable gratitude to my advisor Dr. Aman Dekebo for his immense support .The unaffordable practical support given by Dr. Yadessa Melaku.

I would like to pass my deep appreciation to Dr. Nigussie Megersa, and his coworkers candidates Ato Tshome Tolcha and Alula Yohannes for their excellent support in the GC-MS analysis. A friendly approach and support of prof. Wondimagegn Mamo, other members Department of chemistry AAU staffs for the NMR, UV and IR and other services like digital library and science library. I really valued the cooperative approach of Prof. Ensermu Kelbesa, Prof. Zemedem Asfaw, Prof. Sebsebe Demissew and all members of the AAU herbarium

It is a great pleasure to thank my great friends, fellow students and my lab colleagues including my instructors Dr. Hailemichael Tesso, Dr. Tegene Dessalegn, Dr. Teshome Abdo, Dr. Gellana W/Michael, Ato Worku Dinku and other members of the Applied chemistry program of ASTU and all ASTU staffs helping me on my research work

I would like to thank Yekatit-12 preparatory school administration for their unforgettable cooperation during my research work.

I would like to thank the late Memihir Mitiku Alef my grandfather who is a key person in precluding me to the study of natural products and I really don't put aside a knowledgeable and wise mother Seblewongel Mitiku for a similar contribution and support. I am grateful to acknowledge the support of sisters W/o Almaz Demissie and Dr. Hamer Demissie and brothers Leak-Mariam Demissie in editing my written Materials and Abiy Demissie for that he providing a laptop and my son Ermias Araya for his IT support.

Last but not least I would like to thank my wife Simegnish Teklay and our children for their love and all rounded support.

Table of contents

STATEMENT OF THE AUTHOR.....	II
DEDICATION-----	III
ACKNOWLEDGMENTS-----	IV
LIST OF ABBREVIATIONS-----	VII
ABSTRACT-----	X
1. INTRODUCTION -----	1
1.2. Justification of the research-----	1
1.3. Significance of the research-----	2
2. LITRATURE SURVEY -----	2
2.1. Genus of <i>Dicliptera</i> -----	3
2.2. <i>Dicliptera laxata</i> C.B Clarke -----	4
2.3. Ethnobotanical Background-----	4
2.4 Pharmacological Activities -----	5
2.5 Phytochemical Studies -----	5
2.6 Essential oils-----	14
3. OBJECTIVES -----	15
3.1 General Objective.....	15
3.2 Specific Objectives-----	15

4. MATERIALS AND METHODS	-----17
4.1 Collection and identification Plant material-----	17
4.2 Chemicals and Equipment-----	17
4.3 Methods and procedures -----	17
4.3.1 Extraction of root and aerial part of <i>D. laxata</i> -----	17
4.3.2 Fractionation of the methanol extract (A ₃) of <i>D. laxata</i> -----	19
4.3.3 Extraction of essential oil by steam distillation-----	25
4.3.4 Steam distillation and GC-MS analysis-----	25
4.3.5 Phytochemical Screening-----	27
4.3.6 Isolation and characterization compounds-----	29
4.3.7 Antimicrobial assay-----	30
4.3.7.1 Preparation of inoculums-----	30
4.3.7.2. Preparation of test solution-----	30
4.3.8 Antioxidant Assay -----	31
5. RESULTS AND DISCUSSIONS -----	32
5.1 Analysis of the essential oil of <i>D. laxata</i> -----	32
5.2 phytochemical screening test-----	33
5.3 characterization of compound 1 (from fraction 9C)-----	34
5.4 Partial characterization of compound 2 -----	37
5.5 Analysis of antimicrobial activities-----	37
5.6 DPPH radical scavenging assay-----	39
6 .CONCLUSION AND RECOMADATIONS -----	40
7. REFERENCES -----	42
8. APPENDICES -----	46

LIST OF ABBREVIATIONS

UV	Ultraviolet
IR	Infrared
NMR	Nuclear Magnetic Resonance
TMS	Tetra Methyl Silane
DEPT	Distortion less Enhancement by Polarization Transfer
TLC	Thin layer chromatography
HPLC	High pressure liquid chromatography
AOA	Antioxidant Activities
DPPH	2,2-diphenyl-1-picrylhydrazyl
MHA	Muller Hilton Agar
TEAC	Troxol equivalent antioxidant capacity
HAPX	Hemoglobin ascorbate peroxidase activity
PTLC	Preparatory thin layer chromatography
MS	Mass spectroscopy
GC-MS	Gas chromatography-Mass spectroscopy

List of Tables	page
Table 1. Classification of terpenes.....	12
Table 2. Fractionation of the methanol extract with CHCl ₃ :MeOH.....	19
Table 3. The 3 rd Fractionation.....	21
Table 4. The weight of fractions	23
Table 5. The essential oils composition of <i>D. laxata</i> with 70%.....	27
Table 6. Result of phytochemical screening test	33
Table 7. ¹ H and ¹³ C NMR chemical shift (δ) values for compound 1 recorded in CDCl ₃	36
Table 8. Results of antibacterial test.....	37
Table 9. For gram positive (<i>S. aureus</i>)	40
Table 10. For gram negative, <i>E. coli</i>	40

List of Figures

page

Fig.1 Photo of <i>Erect D.laxata</i>	2
Fig. 2 Leaves blade of <i>D.laxata</i> long.....	3
Fig. 3. Generalized structure of flavonoid.....	7
Fig. 4Triterpene class.....	8
Fig.5. Steroid class.....	8
Fig 6. Steroid alkaloid class.....	8
Fig .7. Asteroid (R=various side chains).....	9
Fig 8. Biosynthesis of steroids.....	10
Fig 9. TLC of the crude extracts,A ₂₁ A ₂₂ ,A ₃	18
Fig10. TLCofA ₁ ,A ₂ ,A ₃ &Mixtures1&2.....	20
Fig11. TLC of fractions from the second packing.....	21
Fig12.The TLC for 37B-41B.....	21
Fig13.TLC for compound 1 (from fraction 9C).....	21
Fig14.The fifth column packing fractions.....	21
Fig15.TLC for the second compound.....	23
Fig16. The setup of steam distillation.....	26
Fig17. A reddish brown ring.....	28
Fig18.Test for flavonoids.....	29
Fig19. β -Sitosterol structure.....	34

Fig20.Petridish for disc diffusion test.....40

ABSTRACT

The present study was carried out on the phytochemical investigation , antimicrobial and antioxidant activities on the root and aerial parts of Dicliptera laxata (Acanthaceae), the plant is used for traditional medicine, especially avoids debility, headache and coughs.

In the present study, the aerial and root parts of the plant were sequentially extracted with organic solvents. Phytochemical screening of the plant were performed on petroleum ether, chloroform, methanol, chloroform/methanol, and petroleum ether/ethyl acetate the aerial and root parts of crude extracts and showed that the presence of alkaloids, flavonoids, sterols, tannins and saponins compounds that has not been screened previously in plant species might be responsible for the claimed activities by local people. The volatile oil of leaf part of the plant was extracted by steam distillation. GC-MS of volatile oil of the aerial part of the plant showed the presence of 47 essential oil compounds with 70 % qualitative abundance and the % yield of 0.23.

In vitro antibacterial activities tests on crude extracts of the plant were done. There is no significant antibacterial effect on chloroform, methanol, chloroform/methanol, and petroleum ether/ethyl acetate aerial and root crude extracts with 0.5mg &1.5mg using disc diffusion method. The antioxidant activity test conducted on aerial chloroform extract indicated its antioxidant properties with % inhibition with DPPH = 55.4% of 12.5 mg/mL of sample concentration.

Repeated use of solvent extraction followed by column chromatography of the plant aerial and root parts and yielded different compounds. Compound 1 was characterized as - sitosterol and compound 2 was a heterocyclic crystalline compound which was partially characterized using UV, FT-IR and NMR spectroscopy by comparing with literature values.

Keywords:

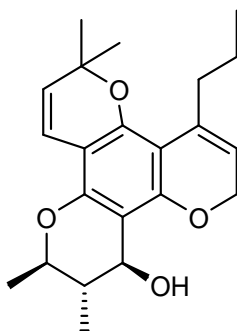
-sitosterol, Dicliptera laxata, Acanthaceae, steam distillation, essential oil, antioxidant and antibacterial

1.1 INTRODUCTION

One of the big challenges of human civilization is getting the proper drug treatment. According to the world health organization (WHO) in 2008 more than 80% of the world population relied on traditional medicine for their primary health care needs. The undesirable side effects and the resistance developed by pathogenic microorganisms demanding the discovery of new drugs extracted from medicinal plants. Enormous plant species from different continent and traditional back grounds are known to be medicinal plants. The identification of these plants needs multifaceted techniques which include biological, phytochemical & molecular ones [1].

In some cases isolating compounds from medicinal plants can be pointless. The herbal treatments by themselves are sufficient since the herbs have their own natural therapeutic and prophylactic values than the extracted drugs. These days herbal medicine is institutionalized for it's at most contribution. The term traditional medicine and complementary/alternative medicine are popular in USA and Europe the sale of herbal products is now over \$100 billion per year [2].

On the other hand isolating the most effective compounds from the traditional medicine is important to avoid unnecessary bulk dosage; which may affect the liver and other organs. The proper dosage for a particular patient must be systematically studied before it is being used. This and other arguments may favor the extraction, isolation and other modern techniques. At the end of the day we can agree both the traditional and the modern techniques have their own pros and cones which made them important in different scenarios. Statistics shows less than 5% of the traditional medicine have been so far analyzed [3]. Examples of drugs dicoverd from medicinal plants; 1, Calanolide A is an anti-HIV drug from *Colophyllum lanigerum* 2003 [1]



There is a lot to be done in the field of searching a curative drug from herbs in Ethiopia. Since there is no organized work in the side of Ethiopian herbal medicine lots of confusions mixed with noble heritages to be systematically studied. In Ethiopia long history traditional medicinal plants for combating various ailments can be confirmed by referring to the medico-religious manuscripts in the country [4]. One of the big challenges in these manuscripts is relating vernacular names with scientific names of plants [5]. There are many effective Ethiopian medicinal plants for numerous humans and livestock ailments. Some findings indicates these medicinal plants found on south, south west, central , north and northwest part of Ethiopia. However there is lack of data to study the indigenous knowledge on use and management of medicinal plants in Eastern Ethiopia [6]

1.2 Justification of the research

The reason why the phytochemical studies of *Dicliptera laxata* is important is the absence of any reported bioactive compound from this particular plant species eventhough it is known as medicinal plant in many communities in Ethiopia and some African countries. The only work we could trace is a pharmacological research and a preliminary phytochemical screening test [7]

1.3. Significance of the research

This plant species is already used by African communities. So, this basic research has an input in developing the application of *Dicliptera laxata* in food science and medicine. In one or another way it may help for the preparation beverages additives of food materials and drugs

2. LITRATURE REVIEW

2.1 Genus of *Dicliptera* A.L Juss (1807) nom.cons

Dicliptera is one of the known genera in the Flora area of the family Acanthaceae, mainly tropical origin[8]. Members of the genus *Dicliptera* are perennial herbs or shrubs. Stems with young branches distinctly rigid leaves petiolate; blades with entire margins. , usually enclosing the flowers; lobes all equal or sometimes one lobe larger than others *Corolla resupinate*, usually purple/magenta or white, with purple marking inside lower lip

Dicliptera is also a genus of 200 species in both old world and new world tropics.



Fig.1 *D.laxata*ie.Erect up to 1m long,whiteflower Fig..2 Leaves blade of *D.laxata*(12-16cm)
Taken by W/o AlmazDemissie

One of the plants from the genus *Dicliptera* such as *D.verticillata*, used to improve male reproductive function, recent treatment of breast cancer,multiple sclerosis[9] other members of this genus like *D.roxburghiana* and *D.chinensis* are known in chines folk medicine .Many phytochemicals has been isolated from these plant species..

2.2*Diclipteralaxata*C.B Clarke

Taxonomy

It is an angiosperm: Family , *Acanthaceae*.

Genus , *Dicliptera*. Species, *laxata*

Botanical Name: *Diclipteralaxata* C.B Clarke

Diclipteralaxata is scrambling perennial herb, stems creeping and rooting at nodes ascending above, or erect, up to 1m high, and with white flower corolla .This herbaceous plant grows and multiplies very easily in many parts of Ethiopia and stay green at all times of the year. Wet montane(coffee)forest Podocarpus forest 1400-2400m GJ ,SU, WG, IL, KF, SD, BA; Sudan, E Africa, Burundi, Dem. Rep. Congo, Cameroon; Nigeria Fernando Po and Malawi.Extensively used medicinal plant in south Western Shoa(local name *Tibeder*),North WesternShoa (*Tibedera*, Oromo), Hadiya zone (*Omoro*),Around Jimma(*Togo*)[8]

2.3. Ethnobotanical Background

The people of NW&SW Shoa including some communities of Addis Ababa used the hot water extract of the green leaves of *D.laxata*seasoned and buttered traditionally as a drink for rebuilding lost blood especially after child birth some people of A.A use it as a remedy for anemic conditions even it is used to replace tea and also used as cosmetic. Some people from A.A said it can be used to avoid infertility of ladies when it is added in *Gonfo* (porridge) meal and buttered. It is also believed ladies who consume *D.laxata* become beautiful. In SW shoa including around Hadiya Zone red decoction for the treatment of orofacial inflammation.

Menit(Me'en) people in the Bench-Maji Zone used its aerial part for the treatment of headache nasal route of administration. In Southern Uganda is used as a poison antidote[7].

D. laxata is one of the medicinal plants of Southern and Eastern Africa; the Swahili speaking people used for the treatment of chest diseases and in Tanzania used to treat stomachache coughs[10]. It is also one of the economic Africa plants in this respect the roots are used in Meshra El Zeraf, Sudan for dyeing mats[11] The blood red decoction used in Tanganyika, as a remedy for general debility[12]. As I have interviewed one of the most senior botanist, prof Zemedu Asfaw (phD). He told me that about 30 years ago they were attending a lesson given by an Ethiopian Orthodox church herbalist Gelahun Abate, he later wrote a book in Amharic called "Etse- debedabe" "its meaning in Geez is a letter about herbs by the assistance of prof Sebsebe Demisew(phD, a botanist). During his lesson to the scientific community at AAU Faculty of natural Science he often lists up to 7 or even more plants for a single treatment at that time some of the chemistry people said this is a useless class and they quit the class. Regardless of the disadvantages and other possible augmentations these days using a combined drug is an effective treatment for different illnesses. For instance drugs like Amoxicillin, Clarithromycin, Metronidazole and Omeprazole are given together for treating H. Pylori. M. Tuberculosis is also treated in a similar manner. The other serious problem in dealing with vernacular names.

The church herbalists give a name *Telenj* for different species; *Achyranthus aspera*, *Hypoestes forskalii*, *Hypoestes aristata* and *Dicliptera laxata* Abate, G, (1989)[5]. There is a big confusion and challenge in collecting the plant sample as well as getting reference material. The plant name *Dicliptera laxata* has been told to me by Professor Ensermu Kelbesa (PhD, botanist) 12 years ago since I brought the plant to the AAU biology department from my home garden as I did it frequently.

2.4. Pharmacological Activities

Studies reported the aqueous extract of *D. laxata* was found to be a highly potent anti nociceptive material. However MeOH-CHCl₃ and CHCl₃ fractions of *D. laxata* with the same dose (200 mg/kg p.o) did not significantly alter the nociceptive responses associated with the first phase (neurogenic pain) and the second phase (inflammatory pain) of formaline test.[7]

2.5 Phytochemical Studies

In phytochemical studies, the qualitative and quantitative studies of major secondary metabolites and some primary metabolites are conducted. Major secondary metabolites are alkaloids, steroids, flavonoids, terpenoids, phenolics, tannins, saponins, anthraquinone glycosides, cardiac glycosides were reported from the plant. The secondary metabolites play a key role in the survival of species over others since they have a crucial involvement in the interactions among the flora and fauna, because they are biologically active compounds. Their biological activity made them prime interest in the field of medicinal chemistry. Studies revealed antimicrobial (antibacterial, antiviral, antifungal) activities and anticancer activities of secondary metabolites. Some of them used in food industries, in agriculture (as hormones, pesticides etc.) and even as chemotaxonomic markers [13]. Due to its usage as a folk medicine, many phytochemical studies have been carried out on other *Dicliptera* species. For example, compounds like C₁₅₋₃₁ fatty acids, flavonoids, carotenoids, -amino acids, botulin, daucosterol and long-chain aliphatic hydrocarbons, have been isolated from *D. roxburghiana* and *D. chinensis* [14]. The preliminary phytochemical screening of the aqueous extract of *D. laxata* (aerial parts) [7] strongly indicated the possible presence of polyphenolics and saponins, with the absence of tannins and anthraquinones. However, a weak positive result was obtained for carotenoids in *D. laxata*. Since flavonoids, polyphenolics and saponins are some of the traced compounds in the genus *Dicliptera* we will discuss basics about these compounds.

Flavonoids

The name flavonoid originated from a Greek word "Flavus" meaning yellow. This day the term represents all plant pigments having the structure based on a fifteen carbon atoms skeleton arranged in C₆-C₃-C₆ configuration. The general structure is represented as follows [15]

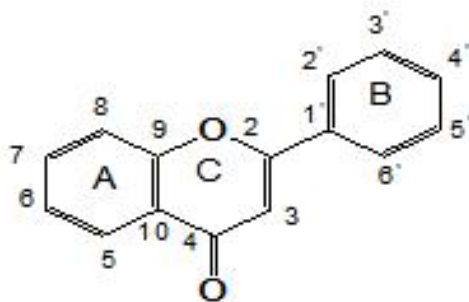


Fig.3 Generalized structure of flavonoid

The variation in oxidation level of the middle (C) ring is the cause of structural differences. Precursors from both shikimate and mevalonate pathways give chalcone which is the first flavonoid unit. Others are obtained by further modification (hydroxylation, methylation, dimerization, bisulfate formation & glycosylation) as the most important part of it. The flavonoids include many of the most pigments which occur throughout the entire plant Kingdom. From the fungi to the angiosperms About 150 glycones are found in both in vegetative parts and in flowers as flower pigments which attracts pollinating birds and insects. Flavonoids are also powerful inhibitors of oxidative phosphorylation. They are also important in food industries, in agriculture, for pest control

Polyphenols

Characterized phenolic structural features, classified based on their source of origin, biological function and chemical structure. According to their chemical structure the followings are available [16].

1. Phenolic acids

Benzoic acids like vanillic acid and gallic acid cinnamic acids such as a p-coumaric acid

2. Flavonoids:

- a) Isoflavones, neoflavoneoids and chalcones
- b) Flavones, flavonols, flavonones and flavanonols
- c) Flavanols and proanthocyanidins
- d) Anthocyanidins

3. Polyphenolic amides:

Avannathramides and capsaicinoids

4. Other polyphenols

Curcumin is a strong anti-oxidant from turmeric. Rosmarinic is a dimer of caffeic acid a tannin ellagic which is a dimer of gallic acid. Hydrolysable tannins are glucose esters of both ellagic acid and gallic acid.

Saponins

Antifungal activities of saponin content are reported in many other species. A saponin is a structure that contains a saccharide portion and aglycone called genin or sapogenine. Based on the type of genin the saponins can be classified into three major classes:

1. Triterpene glycosides
2. Steroid glycosides
3. Steroid alkaloid glycosides

The structures of these three classes of saponins[17]

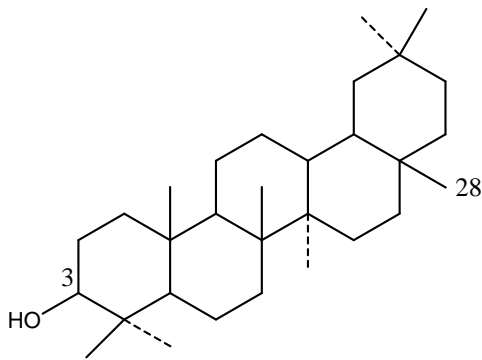


Fig 4 Triterpene class

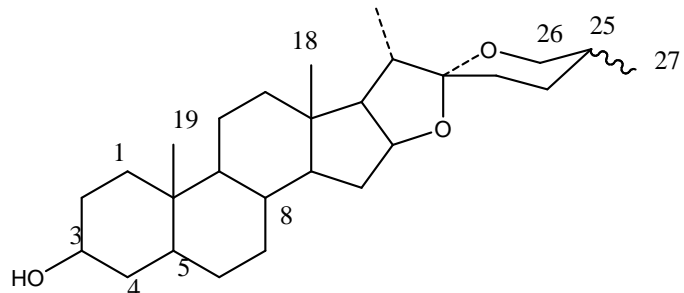


Fig 5 Steroid class

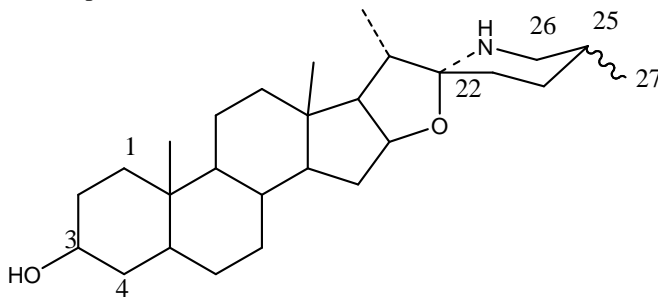
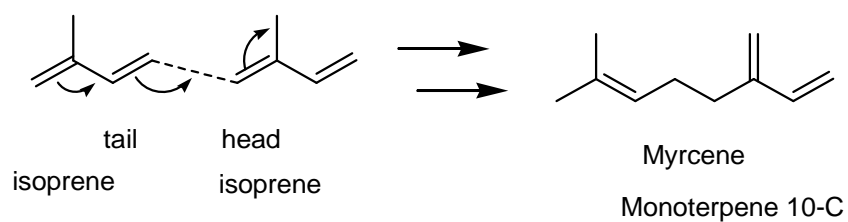


Fig 6 Steroid alkaloid class

Terpenes

Terpenes are essential oils used as medicines, spices and perfumes. They are molecules build up based on the isoprene units joined in head to tail fashion.[18]



A series of such type of reaction creates variety terpenes

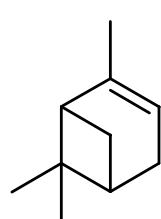
Classification of terpenes

Based on the number isoprene units terpenes have the following classification which can be seen in table 1

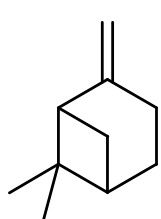
Table 1 Classification of terpenes

	No. of isoprene Units	No. of C-atoms	Group
1	2	10	Mono
2	3	15	Sesquiterpene
3	4	20	Diterpine
6	5	25	Sesterterpene
7	6	30	Triterpene
8	8	40	Tetraterpene
9	>8	>40	Polyterpene

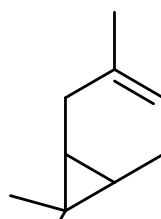
Structures of different monoterpenes were indicated below[19]



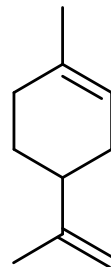
alpha-pinene



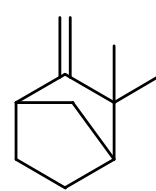
beta-pinene



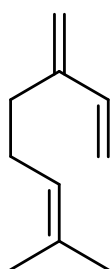
Delta3-carene



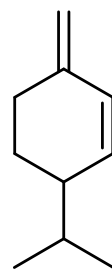
d-limonene



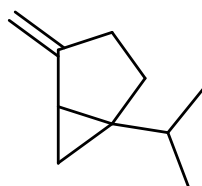
champhene



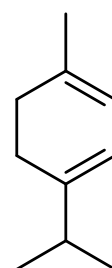
myrcene



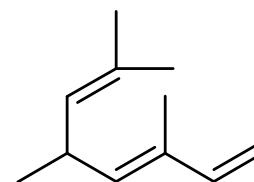
beta-phellandrene



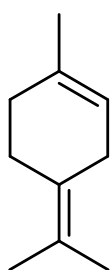
sabinene



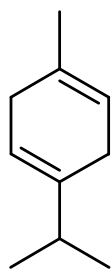
alpha-terpinene



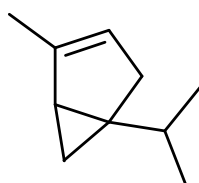
ocimene



terpinolene

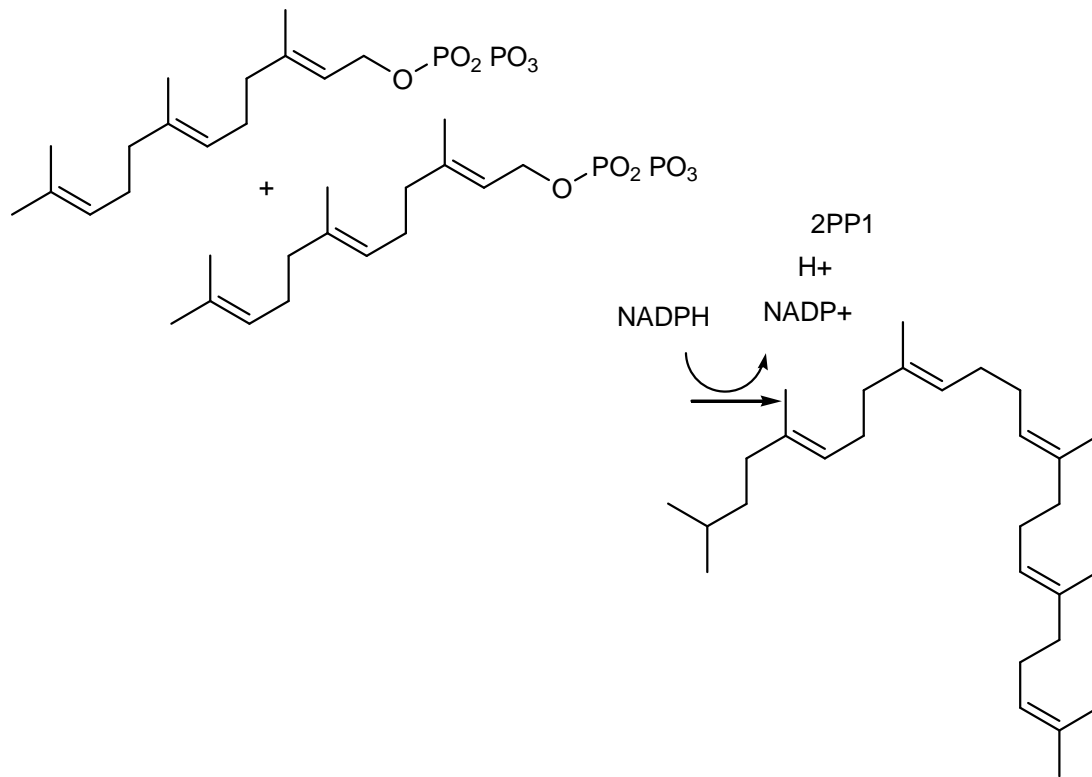


gamma-terpinene



alpha-thujene

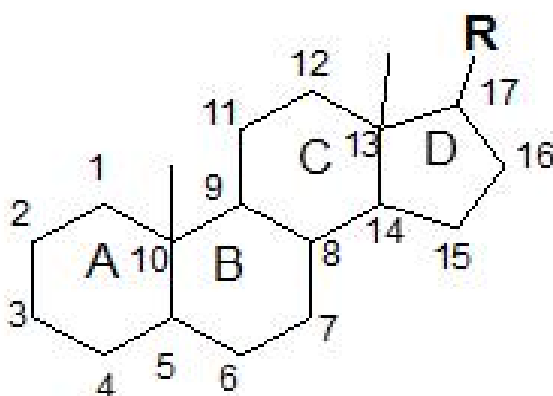
.Two molecules of farnesyl pyrophosphate condense with reduction by NADPH to form squalene by squalene synthase[20]



Squalene, a terpene with C₃₀

Steroids

Since one of the isolated compounds is a steroid it is very important to discuss some basic points about steroids. The lipid extracts of plants and animals contain steroids. The structure is based on tetracycline ring system. Examples: Hormones like androgens(male) and estrogens(female hormone) are sex hormones. Hydrocort is one a regulant of glucose metabolism. Steroids with a –OH group at the 3 position of ring A are called sterols. Zoosterols of animal origin like cholesterol and phytosterols of plant source such as sitosterol, stigmasterol and campesterol. Ergosterol is found in fungi [21]



Eig7. A steroid(R=various side chains)[19]

Biosynthesis of steroids

Steroids are also called heavily modified triterpens e.gs.lanosterol, and cholesterol. They are biosynthesized in living organisms from acyclic hydrocarbons squalenewhich is built up of six isoprene units, that is via ring opening of squalene-2-3-epoxide followed by concerted cyclization.[22]

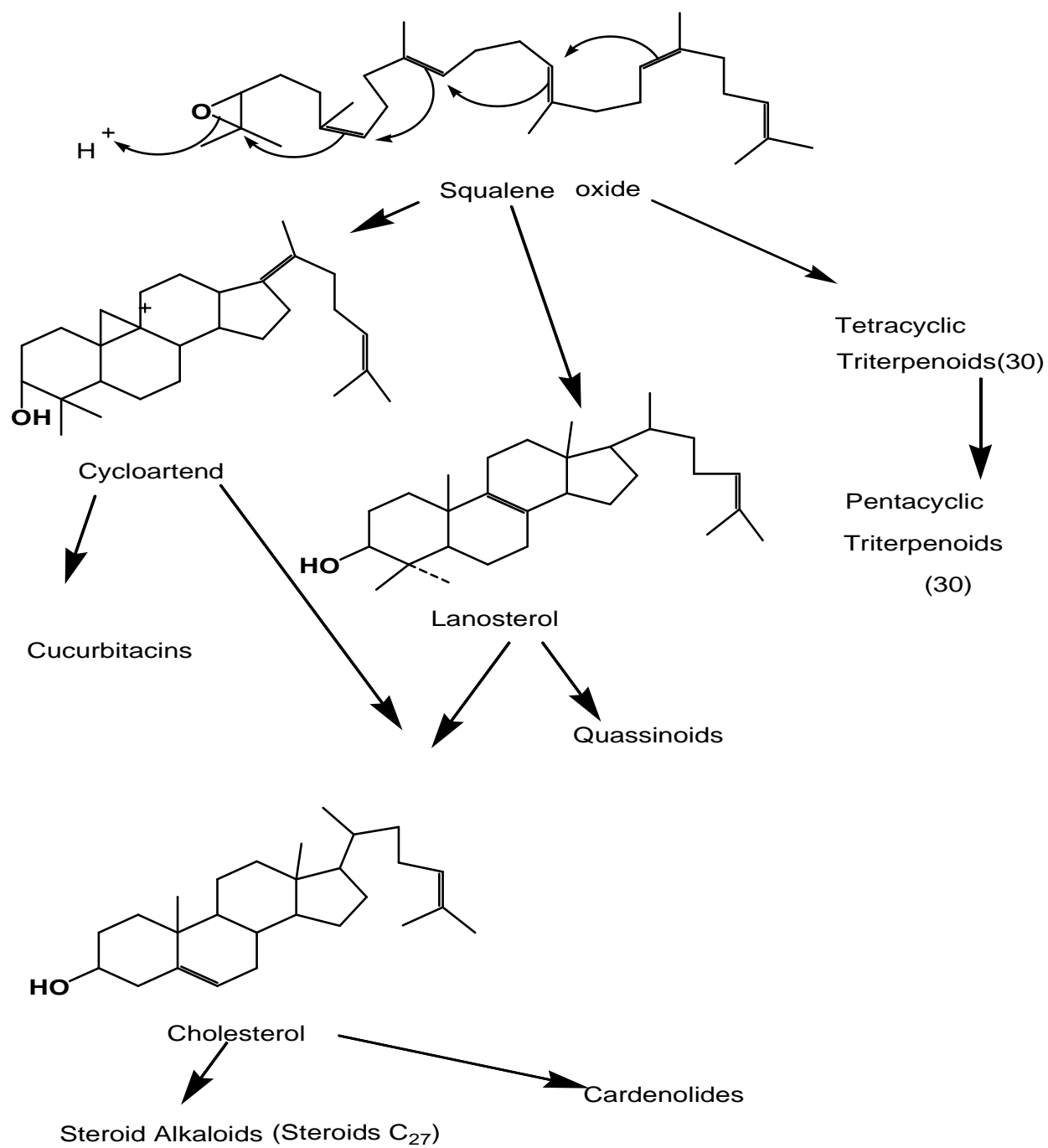
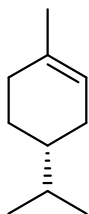
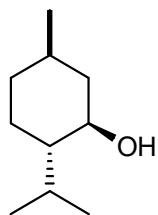


Fig. 8 Biosynthesis of steroids

Terpenoid (isoprenoid): A molecule structurally similar to a terpene, except having a number of carbon atoms that is not a multiple of five or whose skeleton has been modified in some way (oxidation or else)



Limonene is a terpene, derived from biosynthetic combination of two isoprene units.



Menthol is a terpenoid. Its hydroxyl group is not derived directly from isoprene; it must be added by some oxidation process.[23]S

2.6. Essential oils

Since *D.laxta* has significant distinguished smell upon drying, it is due interest to investigate the possible essential oils from *D. laxta*. A fragrant mixture of volatile liquids recovered by steam distillation is known as plant essential oils (ethereal oils). Terpenes and their oxygenated derivatives are the major constituents of essential oils with an immense diversity of structure. Examples: myrcene (oil of bay), α -pinene (oil of turpentine), carvone (oil of spearmint) patchouli alcohol (patchouli oil) These extracts have been used as folk medicines, spices, and perfumes

Some essential oils contain large amount non-terpene components. For example methyl salicylate makes up about 90% of anis oil and eugenol as much as 95% of clove oil [24] Essential oils are extensively used in the manufacture of perfumes toilet soaps, sachets, flavoring materials in candy, ice-cream chewing gums toothpaste, tobacco, shoe polish, printer's ink fish glue alcoholic and non-alcoholic beverages some of them have therapeutic or bactericidal properties Oil composition depends upon both biotic (genetic, ontogenic, morphogenesis) and abiotic (climatic, soil, temperature etc) factors affecting plant growth [24]

3. OBJECTIVES

3.1 General Objective

To isolate and characterize phytochemicals from the root and aerial parts of extracts of *Dicliptera laxata* and study their biological activities

3.2 Specific Objectives

- To extract the plant roots and aerial parts using solvents like petroleum ether, n-hexane, chloroform, ethyl acetate and methanol.
- To conduct phytochemical screening tests on the root and aerial parts extracts of the plant.
- To isolate crude extracts using different chromatographic techniques such as column chromatography and PTLC.
- To extract essential oil from the plant leaves using steam distillation and analyzed using GC-MS.
- To characterize structures of isolated compounds using different spectroscopic techniques such as NMR, IR, UV, and GC- MS.
- To evaluate antimicrobial and antioxidant activities of crude extracts.

4. Materials and Methods

4.1 Collection and identification of the plant material

The aerial part and root of *D. laxata* was collected in November 2015 from different localities of Addis Ababa; Yekatit-12 Preparatory school medicinal plants garden, and from my home garden, Gullele. The sample specimen was identified by Professor Ensermu Kelbesa and deposited at the National Herbarium of Addis Ababa University with a voucher specimen (Araya Demissie 001)

4.2 Chemicals and Equipment

Petroleum ether, n-hexane, ethyl acetate, chloroform, ethanol, methanol, deuterated chloroform (CDCl_3) and deuterated water (D_2O) were used as solvents. Silica gel for column chromatography, vanillin with sulfuric acid for spraying. Digital balance, separatory funnel, Rota vapor, Shaker, Buchner funnel, TLC, TLC jar, Column, filter paper, Ultraviolet lamp, Melting point apparatus, UV spectrometer, IR spectrometer, NMR spectrometer (400MHz, Bruker avance), and GC-MS. Steam distillation setup and anhydrous sodium sulfate, Chemical reagents for phytochemical screening, antimicrobial test, and antioxidant activity.

4.3 Methods and procedures

4.3.1 Extraction of aerial and root parts of the plant

Air dried aerial part (350 g) of *D. laxata* were ground by ordinary mortar and then electrical grinder (blander) and packed in polyethylene bags to avoid entrance of air and any other mixing of surrounding material. 350g of air dried and a powdered the aerial of the plant has been extracted with petroleum ether for 72 h at room temperature. The petroleum extract was filtered using Whatman No. 1 filter paper and concentrated using rotary evaporator under reduced pressure at 40°C until analysis. The marc has been removed and air dried at room temperature. The marc was then extracted with CHCl_3 and MeOH[successively. MeOH extracts of the plant was collected, filtered using what man No. and concentrated by rotary evaporator at 40°C. This procedure was repeated the root part of the plant two times until sufficient crude extract was collected and the crude extract has been kept at 4⁰c until analysis is done.

The following scheme I is used for extraction of the areal part of the plant.

Scheme I. Extraction of areal part of *D. laxata*

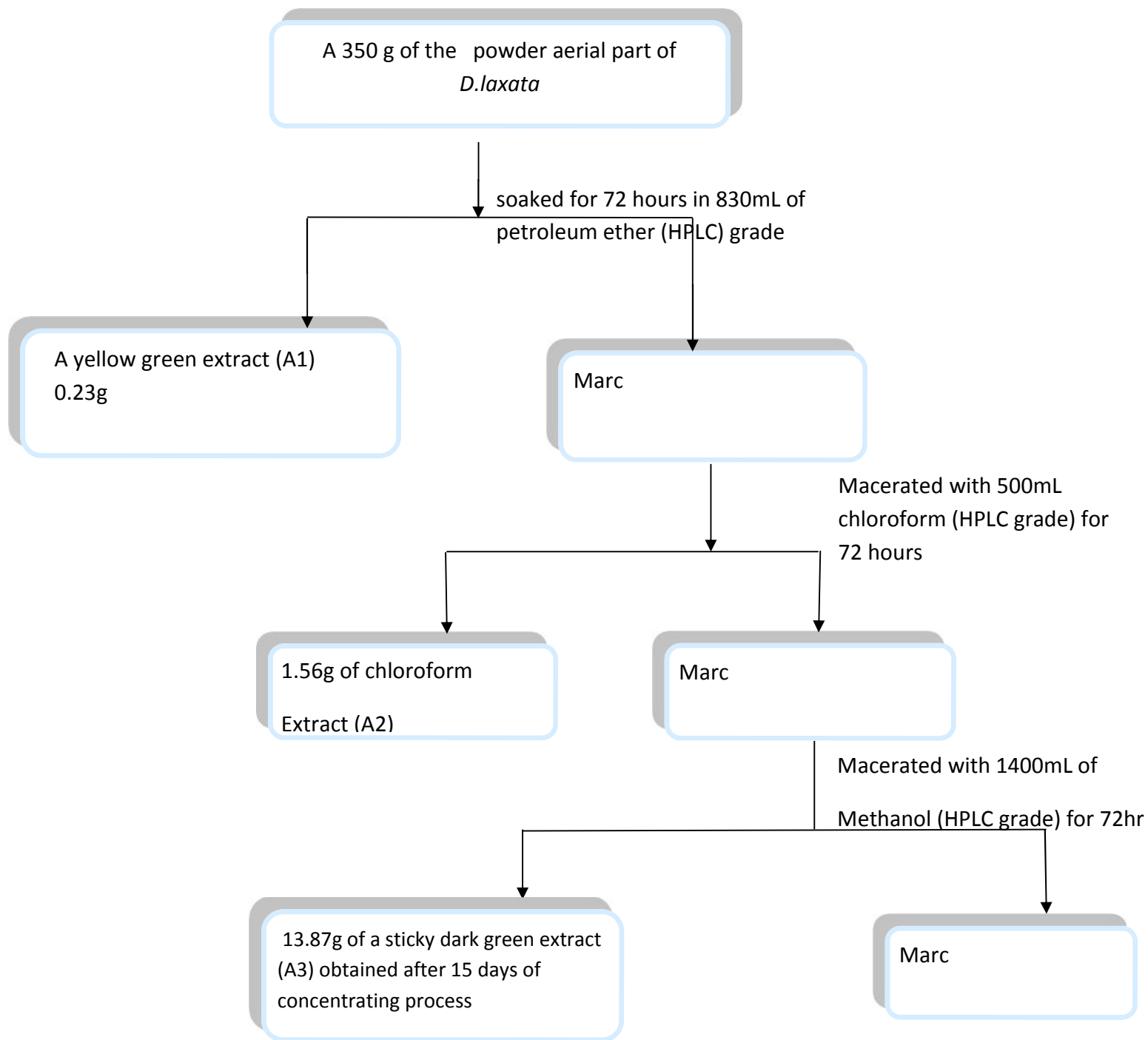




Fig9. TLC of the crude extracts, A₂₁, A₂₂, A₃ (CHCl₃:MeOH, 9.5:0.5)

A₂₁, A₂₂ and methanol extract A₃, using mobile phase chloroform/methanol; 9.5:0.5 proportion

The methanol extract, A₃ exhibit more than 11 visible spots. That is an appreciable amount for further fractionation using column chromatography

4.3.2 Fractionation of the methanol extract (A₃) of *D. laxata*

4.3.2.1 The first fractionation of the methanol extract

A 13.23g of A₃ was adsorbed on silica gel using 120 mL and 18 g of silica gel. The mixture heated on water bath. The column packed with n-Hexane. 150g of silica gel used for packing. The concentrated adsorbed extract was then subjected to the top of packed silica gel column. The column was eluted with increased polarities of n-hexane, CHCl₃ and MeOH solvent systems and 20 fractions were collected by eluting with 50:50 compositions of chloroform/n-Hexane, then chloroform/methanol (both HPLC standards)

Table 2. Fractionation of the methanol extract with chloroform: MeOH solvent system

Ratio of solvents	Volume	Fractions and their code
9.8:2	100 ml	21A-24A
9.7:3	100 ml	25A-29A
9.6:4	100 ml	30A-33A
9.5:5	100 ml	34A-37A
9:1	500 ml	38A-60A
8:2	300 ml	61A-89A
7:3	200 ml	90A-93A

5:5	200 ml	92A-115A
4:6	100 ml	116A-125
3:7	300 ml	126-129/2
2:8	200 ml	130-140
1:9	200 ml	141A-146
0:10	200 ml	147A-158



Fig.10 TLC of A₁,A₂,A₃ & Mixtures 1&2, (Pet.Ether:EtOAc 8.5:1.5)

Fractions 1-12 exhibit one yellow spot using different solvent systems such as petroleum ether/ethyl acetate, and chloroform/methanol mixtures used for TLC. Fractions 44A to 87A were combined to give C₁ because they have similar TLC profile.

4.3.2.2 Isolation of compound 1 (The second and the third fractionations, (cc₂ & cc₃))

The combined fractions dissolved in chloroform dried to 0.634g and fractionation has been conducted with the medium size column. Then eluted with Petroleum ether/ EtOAc solvent system with increasing polarities to yield 116 fractions among these fractions 79B and 80B are likely to have the best single spot with green color even after applying vanillin sulfuric acid and heating it above hot plate with 2cm distance and fractions 97B and 98B are also exhibit similar condition except for their different R_f value. There were also a group green spots with another R_f value the third and the smallest one for fractions between 55B-65B. All these green spots checked by UV by partially blocking the entrance of light by two hands and watching red color through the openings between your hands indicates the existence of chlorophyll. This observation confirms the presence three different types chlorophyll structures in the above

The second combination was done by combining 6 fractions (from 36B-41B) to give C₂. Then using this C₂ the third column packing (cc₃) conducted by the smallest column 16 fractions were obtained.

Table 3. The 3rd Fractionation

Ratio of PE:EtOAc --mL/--mL (V/V ratio)	Volume: added No. 10mL M.cylinder x 10mL
10/0 (100%)	10mL each
9/1	13 x 10mL
8.5/1.5	3 x 10mL
8/2	6 x 10mL
7/3	3 x 10mL



Fig-12. TLC for 37B-41B in 9:1, PE:EA

Among this fractions 9C happen to be a clean purple one spot fraction which can be seen after treating it with vanillin sulfuric acid which invites further instrumental characterization. The following NMR, IR and UV spectra to characterize compound Spectroscopic Determination of fraction 9C was done at Addis Ababa University using 400MHz NMR, with CDCl₃, IR using KBr, and UV the spectral data is available in the next subtopics (5.3)



Fig.11 TLC of fractions from the second fractionation that indicates the presence of compound 1

NB. Another column packing was done by combining fractions 32B and 33B to get a white spotted compound which appeared above purple spot after treating it with vanillin sulfuric acid. Nine fractions 1E-9E. Eventually 5E, 6E and 7E have the same spot with 8C and 10C the R_f

=0.3833 values compared conducting a TLC on the same plate placing the spot of 8C in the middle of the above fractions



Fig.13 TLC for compound 1 (from fraction 9C)PE:EA,9:1, mobile phase,8A,9C,7A,10A

NB. 1A=fraction number.one from the first cc

36B=fraction number 36 from the second c 9C=fraction number 9 of the third cc



Fig.14 The fifth column packing fractions 5E, 6E, and 7E compared with 8C

4.3.2.3 The isolation of compound 2

The other compound is obtained from the 153A and 155A after two & half months of drying. At end of the crystallization process the brown liquid appeared in the upper part that makes easy for decanting it comfortably. Both 153A and 155A treated with 100% petroleum ether. Both of them seem to melt but not dissolved in it. In the next day colorless needle like crystal appeared clearly, then 153A tested with 100% chloroform and creates a milky solution. This may be an evidence for its insoluble property. Again fraction 155A mixed with 7/3(Methanol: chloroform),the crystalline substance seems undissolved ,even it remain undissolved after addition of three folds

of 100% chloroform than added on 153A. Finally a some crystals of these fractions tested with distilled water and it is highly soluble. Then tested 100% of petroleum ether it is insoluble

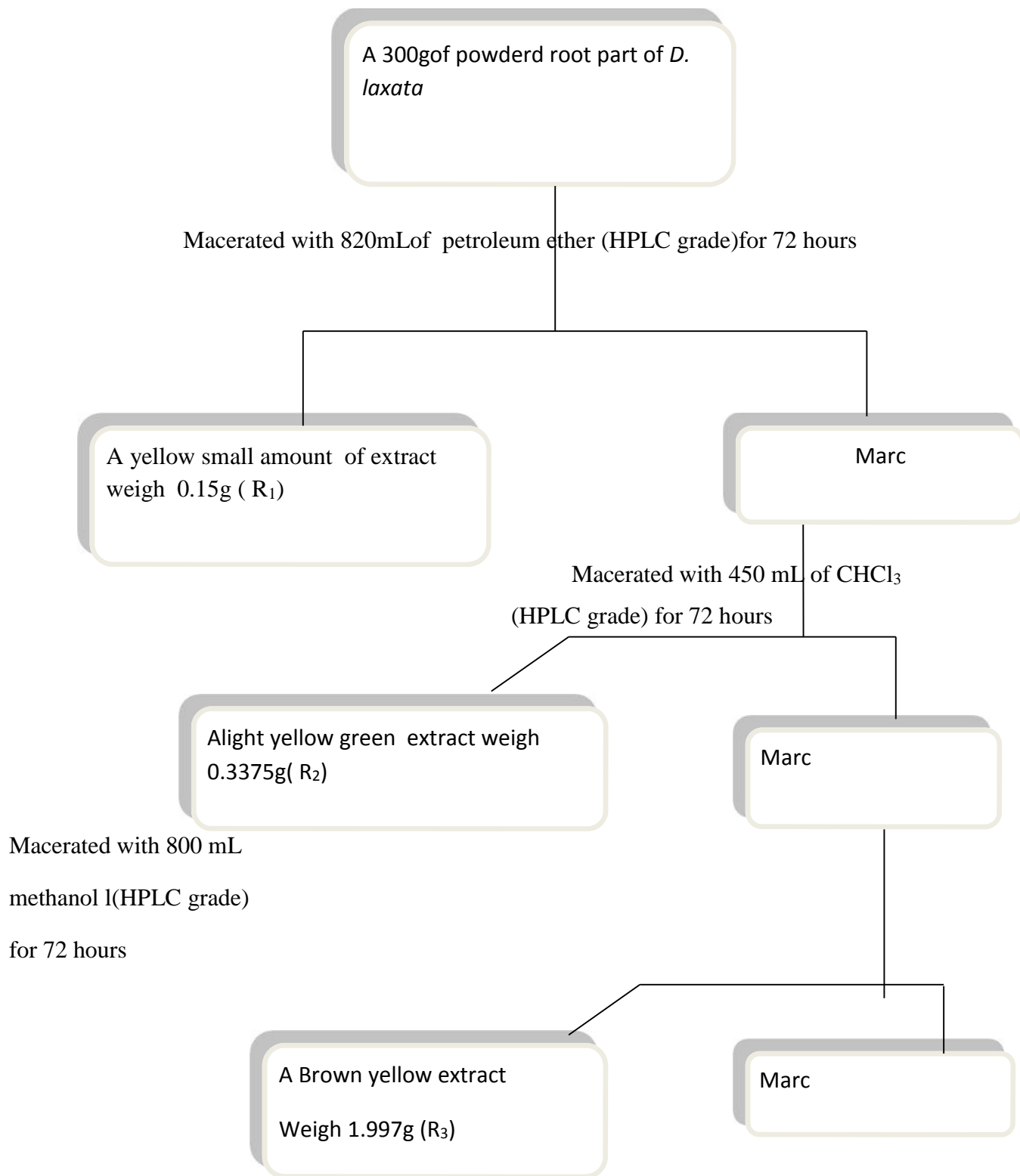
Table4 The weight of factions

Fraction	Mass
153A	103.3mg
155A	751.0mg



Fig 15 TLC for the second compound(155A) compared with sucrose(left)

Scheme II. Extraction of root extracts



4.3.3 Extraction of essential oil by steam distillation

Plant samples for extraction of oils were collected from Addis Ababa 100g of air dried and powdered leaves of *D. laxata* were placed in the 2nd round bottom flask which was then fitted to the 1st flask that contains 400 ml water by a glass connector as shown in fig.16 . The 2nd flask was then fitted with a glass condenser. The 1st flask was and heated using electric stove and the plant was steam distilled at atmospheric pressure for 3 hr. The oil was separated from the aqueous layer by adding 100 ml of chloroform in the separatory funnel. The chloroform layer was then separated from aqueous layer and dried by adding 5 g of anhydrous sodium sulphate and filtered using whatman number one filter paper and concentrated by ordinary evaporation a yield aromatic oil of 0.23g,%yield= (recovered in g/amount treated in g)x100=(0.23g/100g)x100=23%. This procedure was repeated for different leaf samples of *D. laxata* collected from different areas. The oil was then kept at 4°C until analysis.

4.3.4.Steam distillation and GC-MS analysis

Essential oil samples extracted for GC-MS analysis

Experimental of procedures

1. A fresh washed leaves of *Dictyoptera laxata* weighed and placed in a flask then an ordinary steam distillation is conducted on it according to the setup on figure 16 in the next page
2. The distillate was mixed with 1/3rd the amount of 99% chloroform, and then separated the organic phase from the aqueous phase by using burette
3. Sodium sulfate,Na₂SO₄ added on the organic phase and filtered
- 4 The sample was collected and labeled

The arrangements of the set up for steam distillation



Fig.16 The set up of steam distillation(Adama,ASTU)

Experiment I

The 18.7g of *D.laxata* was used

The plant sample collected from my home garden, Wareda 07, Gullele, H.No 543A. There were four similar steps

chloroform extract of the distillate labeled as HG on the top rubber stopper

Experiment II

The 6.5g of *D.laxata* sample was

collected from around Abyot Kirs preparatory school (Gotera A, A)

The chloroform extract of the distillate labeled as AK

Experiment III

The 28.0g of *D.laxata* sample was

collected from W/o Zertihune Mekonnen compound,

Wareda 07, Gullele, H.No 568 Addis Ababa The chloroform extract of the distillate labeled as ZM

Experiment IV

The 18.7g of *D.laxata* sample was used again from my compound, Wareda 07, Gullele the 100mL of distillate mixed with 40mL of chloroform, and then add 5g of sodium

sulfate and filtered. The chloroform extract of the distillate labeled as HG IV

There is a label as the side of the dropper *Dicliptera laxata* on bottle

Experiment V

All samples collected on the above four experiments kept in refrigerator below 4°C till analysis, then injected in the GC-MS instrument.

The working condition of the GC-MS

Technologies,7820 A gas chromatograph equipped with Aligent Technologies,5977E inert MS detector GC separations were carried out on aHP-5ms ultra inert capillary.Helium gas was used as carrier gas at flow rate of 1.0 μLmin^{-1} and data were interpreted using mass hunter Chem-Station. The GC oven temperature was programmed at an initial temperature of 60°C for 2 minutes, then heated up to 190°C at 4°C/min and held at 240°C for 67 minutes. Injector temperature was set at 260°C and detector temperature (flame ionization detector) was set at 280°C using hydrogen gas flowing at 30 ml/min and air flowing at 300 ml/min.

Colum Hp-5ms ultra inert
constant flow

Inlet temp 260°C.
Mode splitless

GC-MS analysis of essential oil of leaf part of *D. laxata*

The oil obtained from the plant was analysed by combined gas chromatography and mass spectrometry. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The identification of the chemical constituents of the oil was determined by their GC retention times and interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) (Adams, 2007).

4.3. 5 .Phytochemical screening

Phytochemical screening test of the crude extracts and isolated compounds from the aerial and root part of the plant were conducted using standard techniques for the detection of alkaloids, flavonoids and sterols [25].

Wagner's Test

KI(2g) and iodine(1.27g) were dissolved in distilled water(5mL) and the solution was diluted to 100mL with distilled water. Few drops of this solution were added to the third test tubes; a brown reddish colored precipitate indicates the presence of alkaloid [25][

Qualitative test for sterols

0.5mL of the extract was dissolved in 5mL of chloroform and equal volume of concentrated sulfuric acid was added by sides of the test tube the upper layer turns red and sulfuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids



Fig 17 Reddish brown ring and upper layer pink(light purple red)

Salkoski Test

0.5g of the extract weighed to test tube is was dissolved in 2ml of chloroform.Sulfuric acid is then carefully added by dropper to form a lower layer. A reddish-brown color at the interface indicated the presence of asteroidal ring (i.e. aglycone)[25]

Potassium Hydroxide Test for Tannins

In to 10 ml of freshly prepared 10% potassium hydroxide (KOH) in a beaker, add 0.5 g of extract was shaken to dissolve. A dirty precipitate was observed which indicates the presence of tannin (Patil and Paikrao, 2015)

Detection of Steroids and Triterpenoids(Liebermann Burchard Test)

The extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube using dropper and boiled in a water bath and rapidly cooled in iced

water. Concentrated H_2SO_4 (2 mL) was added alongside of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red color indicates the presence of triterpenoids [25]

Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids .



Fig 18 Results for flavonoid test from left to right R_1^+ , A_5^- , A_4^- , A_1^+ , A_2^+ , A_3^+

4.3.6. Isolation and characterization compounds

The crude extract was analyzed by TLC to choose best solvent system for column chromatography. Then the crude extract was subjected to column chromatography over silica gel and by increasing polarity of the solvent systems. Different fractions were collected and concentrated over rotary evaporator. The purity of the fractions collected were monitored by TLC and UV lamp. The spot also detected by their UV fluorescence and by spraying Sulphuric acid (vanillin). 1D-NMR (^1H -NMR, ^{13}C -NMR, DEPT), IR and UV has been used to characterize the isolated compounds.

4.3.7 Antimicrobial assay

Crude extracts (chloroform, methanol, MeOH/CHCl₃(7:1), and ethyl acetate/petroleum ether (5:4)) of root / aerial part extract of *D.laxata* their fractions and isolated were evaluated in vitro for antimicrobial assay by using the disc diffusion method. The antimicrobial activities of all samples were tested against gram positive bacterium *Staphylococcus aureus* (*S.aureus*) and gram negative bacterium *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*Pseu.ae*) using MHA medium. The antimicrobial test was conducted in the laboratory at Oromia Public Health Research Capacity Building & Quality Assurance Laboratory Adama (Ethiopia.)

4.3.7 .1 Preparation of inoculums

The test bacterial strains were transferred from the stock cultures and streaked on Mueller Hinton plats and incubated for 24 hrs. Well separation bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved MHA that were cooled to about 45°C and mixed water bath and gently swirling the flasks. The medium were then poured to sterile Petri plats, allowed to solidify and used for the biotest called disk diffusion bioassay.

4.3.7.2. Preparation of test solution

1.5 mg and 0.5 mg of the crude extracts and fractions were dissolved in Chloroform with 0.3mL and 0.1mL, respectively.

4.3.8 Antioxidant Assay

DPPH bleaching assay

The free radical scavenging activity of chloroform crude extract was evaluated according to previously described methods [26]. Briefly, 25mg/mL chloroform extract of *D. laxata* was dissolved in methanol solution of the DPPH. The DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was added in the quartz UV-Vis cuvette. The final concentration of DPPHs in the cuvette was 40µg/mL used as positive control. The process was monitored spectrophotometrically at a fixed wave length (517nm) and DPPH was consumed until this point. The absorbance of the sample compared with DPPH bleaching was determined [26].

An experimental procedure for the antioxidant activities

A chloroform extract of aerial part *D.laxata* (12.5 mg) mixed with 1 mL of chloroform. The Chloroform solution of extract with concentration 12.5mg/mL mixed the with methanol solution of DPPH that is prepared by dissolving 4 mg DPPH in 100 mL methanol using 100 mL volumetric flask. The violate color of the DPPH solution disappeared instantly. Then plant sample was placed in oven for 30 min. A blank was a solvent grade methanol. The wave length adjusted at 517nm and the absorbance of the standard was recorded at 0.927nm. The absorbance of the sample measured as 0.414nm.

5.RESULTS AND DISCUSSION

5.1 Analysis of the essential oil of *D. laxata*

D. laxata (1 00 g) was steam distilled for 3 h in a volatile oil yielded 0.23% colorless oil.

The total ion chromatogram was obtained and showed in Appendix 14. Twenty two compounds were identified representing approximately 72.45% of the oil (Table 6). There were also unidentified compounds with no matches found in the libraries (14.97%). The major volatile components were identified as Eicosane (7.64%), Hexacosane (7.09%), Nonadecane (7.00%), 2-methyloctacosane (6.84%), (E)-3-Eicosane (5.96%), Tetracosane (5.41%), Pentadecane (5.17%), Tritetracontane(4.76%), **Heptadecane**(4.86%), **Octadecane** (4.32%).

Table 6 Composition of the leaves essential oil of *D .laxata* (sample HG IV, App 14)

No	Compounds	RT	Area %
1	Unidentified	4.9	0.39
2	Unidentified	4.96	0.38
3	Styrene	7.1	0.80
4	(1R) -2,66 Trimethylbicyclo [3.1.1]	8.7	0.51
5	Unidentified	10.7	0.97
6	Octacosane	41.9	1.75
7	Dodecane,2-methyl	45.8	1.6
8	Unidentified	45.8	2.2
9	Decane,3,8-dimethyl	50.9	2.4
10	Unidentified	50.9	1.95
11	Heneicosane	58.1	3.01
12	Dodecane	59.2	1.48
13	Tetratetracontane	61.0	3.95
14	Unidentified	63.2	1.09
15	Tetracosane	63.7	5.41
16	Unidentified	64.7	3.60
17	2-methyloctacosane	66.3	6.84

18	Hexacosane	68.9	7.09
19	Tritetracontane	71.3	4.76
20	Pentadecane	73.7	5.17
21	(E)-3-Eicosene	75.9	5.96
22	Nonadecane	78.2	7.00
23	Unidentified	78.8	1.52
24	Eicosane	80.3	7.64
25	Unidentified	80.7	0.87
26	Heptadecane	82.5	4.86
27	Octadecane	85.1	4.32
	Total		87.42

RT=retention time

5.2. Phytochemical screening

Phytochemical screening tests were conducted on the areal and root solvent extracts of *D. laxata* using using standard procedures and indicated the presence of alkaloids, flavonoids, steroids, tannins and saponins in the plant (Table 7).

Table. 7 Result of phytochemical screening

Phytoconstituent	Test	A ₁	A ₂	A ₃	R ₁	R ₂	R ₃	MeOH/Ch;7:3A ₄	PE/EA;1:1 _{A5}	BRD	C ₁	C ₂
Alkaloids	Wagner's test	+	+	+	+	+	+	+		-		-
Flavonoids	Qualitative test	+	+	+	+			-	-			
Sterols	Qualitative test for steroids	+	+		+	+	-	+	+		+	-
	Liebermann Burchard test							+				
Tanins	Potassium hydroxide test	+	+	+	-	-	-	+	+			
Saponins	Foam test	-	-	+	-	-	-					

Key: for the above table A=aerial, R=root, PE=petroleum ether, EA=ethyl acetate

Ch=chloroform, BRD=blood red decoction (subscripts 1,2&3 stood for PE, chloroform & methanol respectively .e.g. A₁=petroleum ether extract of the aerial part.C₁ and C₂ are compounds **1** and **2** respectively

A reddish brown ring observed for compound **1**, the upper layer is red and the lower yellow showed the presence of steroids (Refer Fig 17)

5.3. Characterization of compound **1**

Compound **1** is a white amorphous solid and exhibits a positive result for a qualitative test for steroids. TLC analysis shows a single spot indicating the compound is pure. IR spectroscopic analysis of the compound (Appendix 4) showed absorption bands appeared at 3570.36 – 3186.51 cm^{-1} that is characteristic of O-H stretching, 2842 and 2940 cm^{-1} are due aliphatic C-H stretching, 1625 cm^{-1} due to double (C=C) stretching, 1055 cm^{-1} due to (C-O). The absorption frequency at 731 cm^{-1} signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for β -sitosterol.

The ^1H NMR spectrum (Table 7, Appendix 1) of compound **1** showed the presence of six methyl signals that appeared as two methyl singlets at 0.68, and 1.01; three methyl doublets that appeared at 0.81, 0.83, and 0.93; and a methyl triplet at 0.84. The ^1H NMR spectrum of compound **1** also showed one olefinic proton at 5.44. The proton NMR showed the oxygenated proton H-3 appeared as a multiplet at 3.44.

The ^{13}C -NMR spectrum of the compound (Table 7, Appendix 1) has shown recognizable signals 140.7 and 121.7, which are assigned C5 and C6 double bonds respectively. The value at 23.9 corresponds to angular carbon atom (C19). ^{13}C NMR and DEPT (Appendix 3) spectra showed the presence of twenty nine carbon signal including six methyls, nine methylenes, eleven methine and three quaternary carbons. Based on the above spectroscopic data and comparison of these data with those reported in the literature [27] the structure of the compound was proposed to be β -Sitosterol.

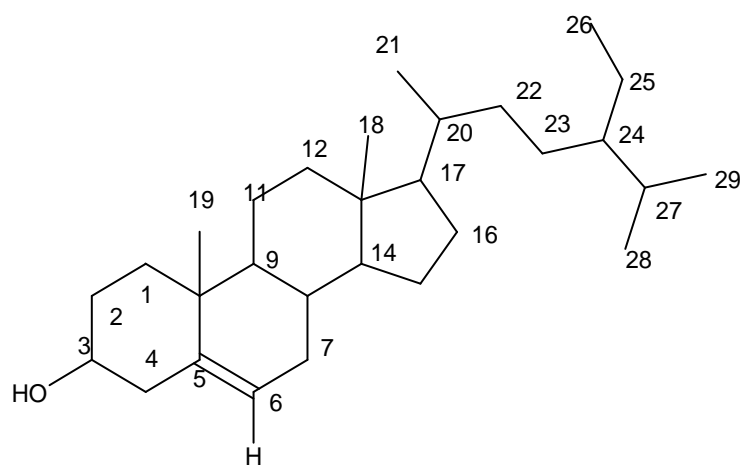


Fig.19 Proposed structure of compound **1** (-Sitosterol)

Table-7 ^1H NMR and ^{13}C NMR chemical shift (δ) values for compound **1** recorded in CDCl_3 and compared with literature[27] values.

Position	$^{13}\text{C}\delta$ literature	$^{13}\text{C}\delta$ Compound 1	DEPT-135	$^1\text{H}\delta$ Literature	$^1\text{H}\delta$ Compound 1	Multiplicity, Number of hydrogen
1	37.5	37.3	CH_2		2.36	t, 2H
2	31.9	29.7	CH_2			M
3	72.0	71.85	CH	3.53	3.44	m
4	42.5	42.3	CH_2		2.36	d, 2H
5	140.9	140.7	C	-	-	-
6	121.9	121.7	CH	5.37	5.436	T (J = 6.4 Hz)
7	32.1	31.6	CH_2			t, 2H
8	32.1	31.9	CH			m, 1H
9	50.3	50.1	CH			m, 1H
10	36.7	36.5	C	-		-
11	22.3	22.2	CH_2			m, 2H
12	39.9	39.8	CH_2			t, 2H
13	42.6	42.3	C	-		-
14	56.9	56.8	CH			m, 1H
15	27.7	28.3	CH_2			m, 2H
16	27.3	27.2	CH_2			m, 2H
17	58.3	56.9	CH			M
18	20.7	19.8	CH	1.01	1.03	s,3H
19	23.2	23.1	CH_3	0.68	0.701	s,3H
20	36.1	36.1	CH	1.64		m, 1H
21	19.4	19.4	CH_3	0.93	1.06	s,3H
22	33.9	33.9	CH_2			m, 2H
23	29.9	29.7	CH_2		1.25	m, 2H
24	46.1	45.9	CH		1.46	m, 1H
25	25.8	26.1	CH_2			m, 2H
26	12.2	11.9	CH_3	0.84	0.951	t,3H
27	31.7	37.3	CH			m, 1H
28	21.0	21.1	CH_3	0.83	0.83	d (J=6.4Hz),3H
29	21.0	21.2	CH_3	0.81	0.81	d (J=6.4Hz),3H.

5.4 Partial characterization of compound 2

Compound 2 is a very clean needle like crystals, Mp 289°C. Its IR spectroscopic data indicated absorption bands at 3442 cm⁻¹ shows OH, 2405.5 a triple bond, and 1380 cm⁻¹ C=C bending UV at lambda max at 303.38. The ¹³C NMR at δ 169.36 ppm, 66.21ppm, and two carbons at 53.4ppm. Its DEPT has no signal. Therefore ¹³C NMR, ¹H NMR and DEPT indicate four carbon atoms and all carbons are quaternary carbons Appendix 6,7&8

The IR and UV spectra for Compound 2 revealed the following results about the compound. All information's written above are not enough to predict the structure unless it is supported by elemental analysis and mass spectroscopy to fully characterize its structure.

5.5 Analysis on antimicrobial activities

The effects of eight extracts A₂, A₃, A₄, A₅, R₂, R₃, Da and Df on three organisms S.a, E.c & Pseu.a were studied and shown in Table 8. Appendix 15

Type of extract	Extract concentration in mg	<i>S. aureus</i> Gram (+) Zone of inhibition	<i>E. coli</i> Gram (-) Zone of inhibition	<i>Pseu.a</i> Gram(-) Zone of inhibition
A ₂	1.5	6	6	
	0.5	6	6	
A ₃	1.5	6	6	
	0.5	6	6	
A ₄	1.5	6	6	
	0.5	6	6	
A ₅	1.5		6	6
	0.5		6	6
R ₂	1.5	6	6	
	0.5	6	6	
R ₃	1.5	6	6	
	0.5	6	6	
Da	1.5	11	12	11
	0.5	6	6	6
Df	1.5	15	6	6
	0.5	6	6	6

Control for

Pseudomonas.a

6mm

S.aureus

6mm

The standard antibiotics again tested in the same situation as follows

Table 9. Results on antimicrobial activities of standard antibiotics against gram positive (*S.aureus*) bacteria as evaluated in the same laboratory condition

Antibiotic	Code	Conc <i>mg</i>	Acceptable limits	Zone inhibition in mm
Cefoxitin	Fox	0.03	23-29	26
Clindamicin	Da	0.01	24-30	27
Erythromycin	E	0.015	22-30	25
Penicillin	P	0.01	26-37	30

Table 10. Results on antimicrobial activities of standard antibiotics against gram negative (*E.coli*) bacteria as evaluated in the same laboratory condition

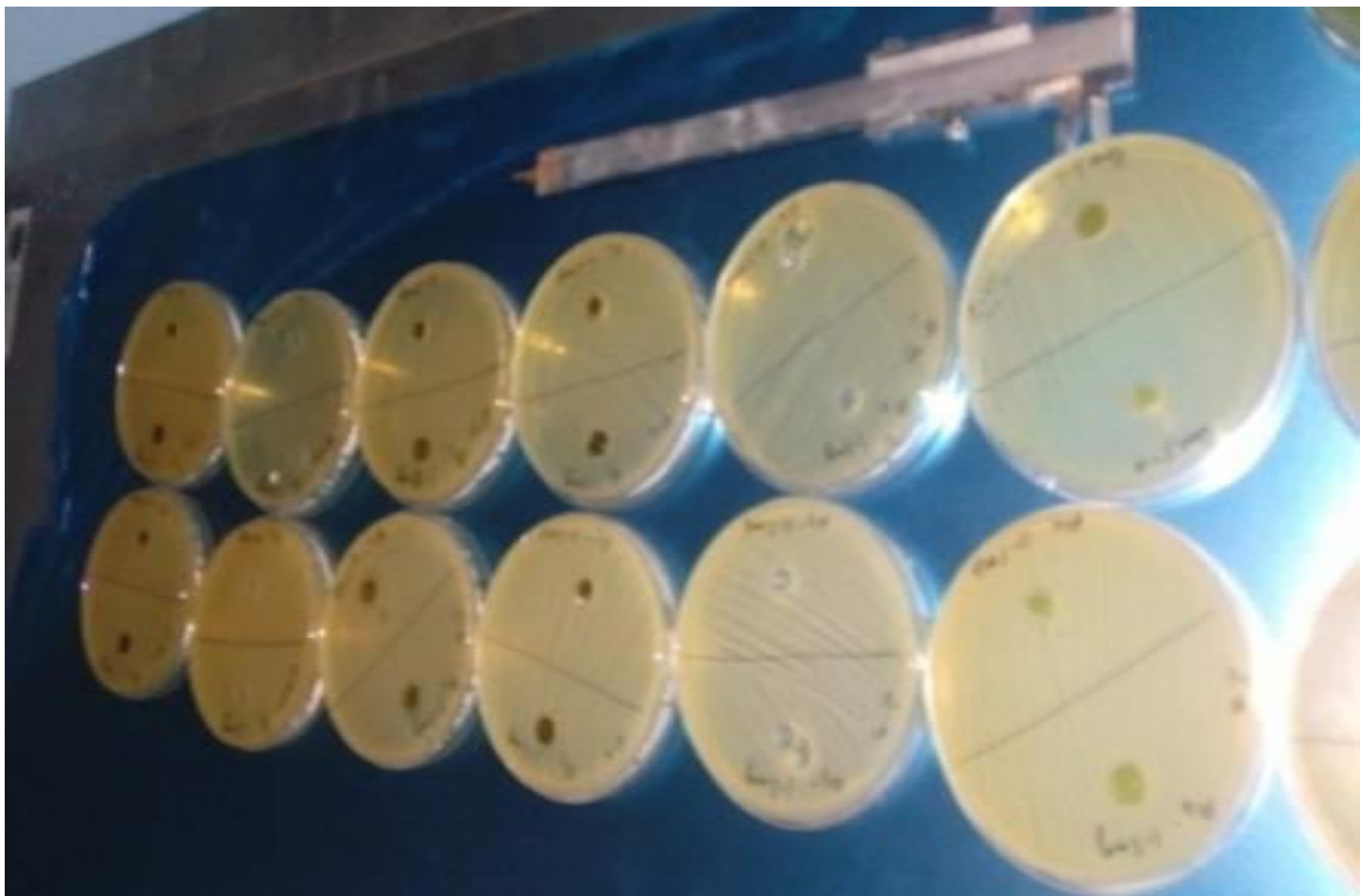
Ampicillin	AMP	0.01	16-22	22
Amox-clavacid	AMC	0.03	18-24	19
Ceftriaxone	CRO	0.03	29-35	30
Ciprofoxacin	CIP	0.005	30-40	31
Gentamicin	GM/CN	0.01	19-26	20

The last two extracts were checked for other plant extracts treated in the same conditions written *here* for comparison with *D. laxata*. The methanol extract of *Dodonea angustifolia* (Da) (as extracted by Chala Bedada) was moderately active for the three organisms

S.aureus, *E.coli* & *Pseu. aeruginosa*. when it is compared with standards like penicilline (30 mm

and similarly ethyl acetate extract of *Carissa spinarum* L.(Df) (as extracted by Dessalegn Feyissa) was even more moderately effective than Da (Chala) for *E. coli*. According to Oromia public health research, capacity building & quality assurance Laboratory

Whereas the antibacterial activity for A₂, A₃, A₄, A₅, R₂, & R₃ was 6 mm for all extracts that is the same as the original diameter (Control). Therefore these crude extracts do not have significant antibacterial effect.



Fig, 20 Petri dish for disc diffusion test

5.6 DPPH radical scavenging assay

The following absorbances are recorded at constant wavelength, 517 nm based on DPPH bleaching assay for 12.5 mg of the chloroform extract of *Dicliptera laxata*

Absorbance of the standard (Ab_{standard}) = 0.927 nm and

Absorbance of the sample (Ab_{sample}) = 0.414 nm

Percent inhibition = $(Ab_{\text{standard}} - Ab_{\text{sample}}) / Ab_{\text{standard}} \times 100 = (0.927 \text{ nm} - 0.414 \text{ nm}) / 0.927 \text{ nm} \times 100$

$$= 0.5534 \times 100\%$$

$$= 55.34\%$$

The percent inhibition of ascorbic acid is 94% in the same condition. So the 12.5g of chloroform extract of *D.laxata* is moderately antioxidant as it is compared with ascorbic acid.

6. CONCLUSION AND RECOMADATIONS

The herbal medicinal plant *Dcliptera laxata* contains a β -sitosterol a white amorphous substance isolated with 9:1 (Petroleum/Ethyl acetate). The compound was characterized using NMR, IR, UV. β – *Sitosterol*, Compound **1** can be used to avoid debility as it was mentioned in the previous literature reports. The compound was also reported to have antibacterial ant diabetic and immunity booster activities [28].The antibacterial test must be done for the pure compound to confirm these activities. It also blocks cholesterol absorption sites in human intestine [29].Based on these information compound **1** can be a potent a versatile drug for various ailments. The second compound was colorless needle, isolated from highly polar water soluble fraction was partially characterized. To fully characterize the compound 2D NMR, SEM and XRD data are required.

Twenty one compounds were identified in the essential oils of the leaves of the plant. Since there is no significant antibacterial effect for six crude extracts A₂, A₃, A₄, A₅, R₂, & R₃ in this work it is required to check their antibacterial activities with higher concentrations. It is also important to evaluate antibacterial activities for water extracts and for the isolated compounds. Additionally, antifungal activity and antiviral activities must be evaluated on crude solvent extracts, fractions and pure compounds. The aerial chloroform crude extract of *D.laxata* is exhibited antioxidant from the DPPH bleaching assay with % inhibition 55.34%. It is the sign for anticancer activity of the extract and needs to conduct anti-cancer assay.

Additionally the following recommendations were made for future work on this plant.

- Other chromatographic techniques such as PTLC and HPLC should be used to isolate more compounds from the plant.
- More spectroscopic techniques such as 2D NMR (HMBC, HSQC, H-H COSY,) and MS should be used to isolate and characterize more compounds from different parts of the plant.

- Further bioassay guided isolation and characterization work should be done to fully characterize bioactive constituents of the plant crude extracts to identify all active compounds against a variety of bacteria and fungi.

References

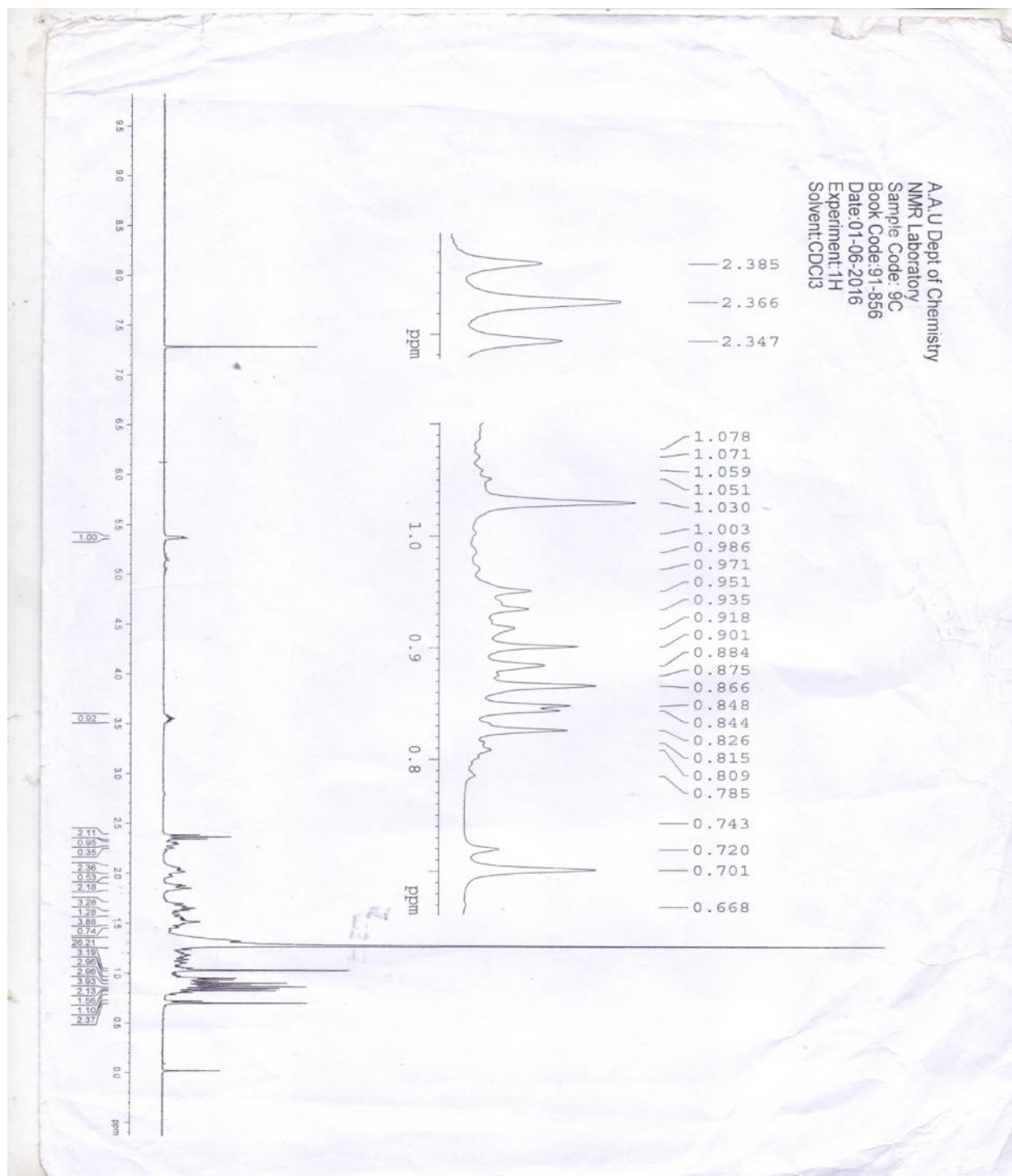
1. Balunas, M.J., Kinghorn D.A., (2005). Drug Discovery from medicinal plants, *Life sciences*, 78, p.431-438
2. Saad, B., Said, O., (2011). Greco-Arab and Islamic Herbal Medicine, p 47, 48. by John Wiley & Sons, Inc, Canada.
3. A., Vohr & Kaar, H., (2011). Chemical Investigation of Medicinal Plant *Ajugabracteosa*, p 37-38, org chem.(NP), Lovely Professional University, Punjab.
4. Luelekal, E., Kelbessa, E., Bekele T and Yeneger H (2008). *Journal of Ethnobiology and Ethnomedicine* 2008, 4; 10, bioblio-hb-53.
5. Abate, G., (1989). Etse-Debidabe (Amh), p132, AAU.
6. Belayneh, A., Asfaw Z., Demissew S., Bussa N F., (2012). Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Woreda Estern. Ethiopia, 2012, 8:44 doi.10.1186/1746-4269-8-42 (<http://www.ethnobiomed.com/content/8/1/42>)
7. Woldemariam, M., Yarlagadda, R., Asres, K., (2013). *In vivo* anti-inflammatory and anti-nociceptive activities of the aerial extract of DL. Dept. of pharmacy Jimma University, Jima Ethiopia. Original Article V (7), Issue 3 p 216-223
8. Hedberg, I., Kelbesa E, Edwards, S., Demissew S, Persson E, editors. (2006). Flora of Ethiopia and Eritrea, Gentianaceae to Cyclohelaceae, Vol. 5. Addis Ababa, Ethiopia. The National Herbarium, Addis Ababa University, p.345, 443-5, 449
9. Massoma, D., Manuel L., Gozales G.F (2014). Synergic effect of the hydroalcolic extract of fragrance tessmanni *Journal List of Pharmacognosy. Res*, V(1) PMC38997015
10. CRC (2012). World Dictionary of medicinal and Poisonous plants. Common Google. <http://books.google.com.et/books?isbn=1482250640> Umberto Quatrocchi—science .
11. Uphof, J.C. Th (1959). "Dictionary of Economic Plants" , p.191, New York Hantner publ. Co And Codictore Wheldo and Wesley.
12. Watt J.M & Breyer Brandwijk, M.G. (1962). "Medicinal and Poisonous Plants of Southren And Eeastern Africa" p.3 E&L Livingston Ltd Endiberg London
13. Torssel K.B.G, (1997). Natural Products Chemistry, p354, Swedish pharmaceutical Society.
14. Malik P. (2014). Structure- Function Elucidation and prooxidative of antioxidative Activities. of poliphenolic compound Curumin. Art. Id 396708, 8 pages.

15. Ahmed., I, (1996). Polyphenolic compounds of four biologically active plant species *Rumex chalpensis*,*R.Nepelesis*....pdf 305H Nepalosis,p 11,49-91,116.Dep't of Chemistry Quazid-I-Azam University Islamabad.
- 16.Guelph(2010). Food Research Center,Agriculture ,andAgri-Food Canada,ChemisMtry,. Biochemistry of Diatery Polyphenols p1231-1246 NIG 5c9,Canada.
- 17.Hastettmann,K.,Maston,A.,(2005). saponins (a)P 1-4 Cambridge University Press UK
- 18.McMury.(1996). Organic chemistry, p1108-1109
- 19.<http://www.google.et/Search?q=terpens&prmol=ivnspb&thm=1sch7tbo=o&source=univ&Sa=x&ved=OahUKEW,Nov.2015AddisAbaba>
- 20.<http://en.wikipedia.org/wiki/squalene>, Nov. 2015 Addis Ababa
- 21.Wikipedia;Ostlund RE, Racette SB, Stenson WF (1 June 2003). "Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ". *Am. J. Clin. Nutr.* **77** (6): 1385–9. PMID 12791614.sept. 2016. Addis Ababa
- 22 Madland,E.,(2013). Extraction, Isolation and Structure Elucidation of Saponins From *Herniaria incana*,p.6.Norwegian University of Science and Technology.
- 23.<http://www.chemuclu.edu/harding/IGOC/T/terpenoid.html>Nov.2015AddisAbaba
- 24.G.yohannes .p (1980). Analysis of essential oils of *Artemesia rehan anymbogoncitratus* p.1,4,AAU
- 25.Santhi,R,G .,Lakshmi A.M.,Priydarchin &L. Anandaraj (2011). Phytochemical screening of *Nerium oleander* leaves *Momordicacharantia* Leaves. *Internationaljournal of pharmacy* **2(1)**:131-135(sterol)
- 26.Kaur,P.,Singh,B. , Kumar,S.,Kaur.S., (2008). *In vitro* evaluation of free radical scavenging activity of *RubiacordifliaL.*,*Journal of Chinese clinical Medicine* March, Georgetown University p.278-284
- 27.Chaturvedulav, s. p and Prakash I.,(2012). Isolation of Stigmasterol and β – *Sitosterol*from dichloro methane extract of *Rubussuavissimus*International current Pharmaceutical journal 2012,1(9):239-242([http:// www.icpjonline com/documents/161 19/03.pdf](http://www.icpjonline.com/documents/161_19/03.pdf)) Atlanta,GA 30313,USA
28. www.sciencesecurejournals.comKaran et al.,(2012) page 11 (2016) sept Addis Ababa
- 29 .<http://www.mdpi.com/1420-3049/19/9/14114/htm> (2016) septAddis Ababa
- 30.[www science direct con](http://www.science-direct.com) *Journal of king suad university-science* July 2015 vol 27(3):224-232 doi:101016/j...oct.2016.Addis Ababa
31. Anderson,Q., R.Markham,K.,(2006).Flavonoids chemistry ,biochemistry&application ©

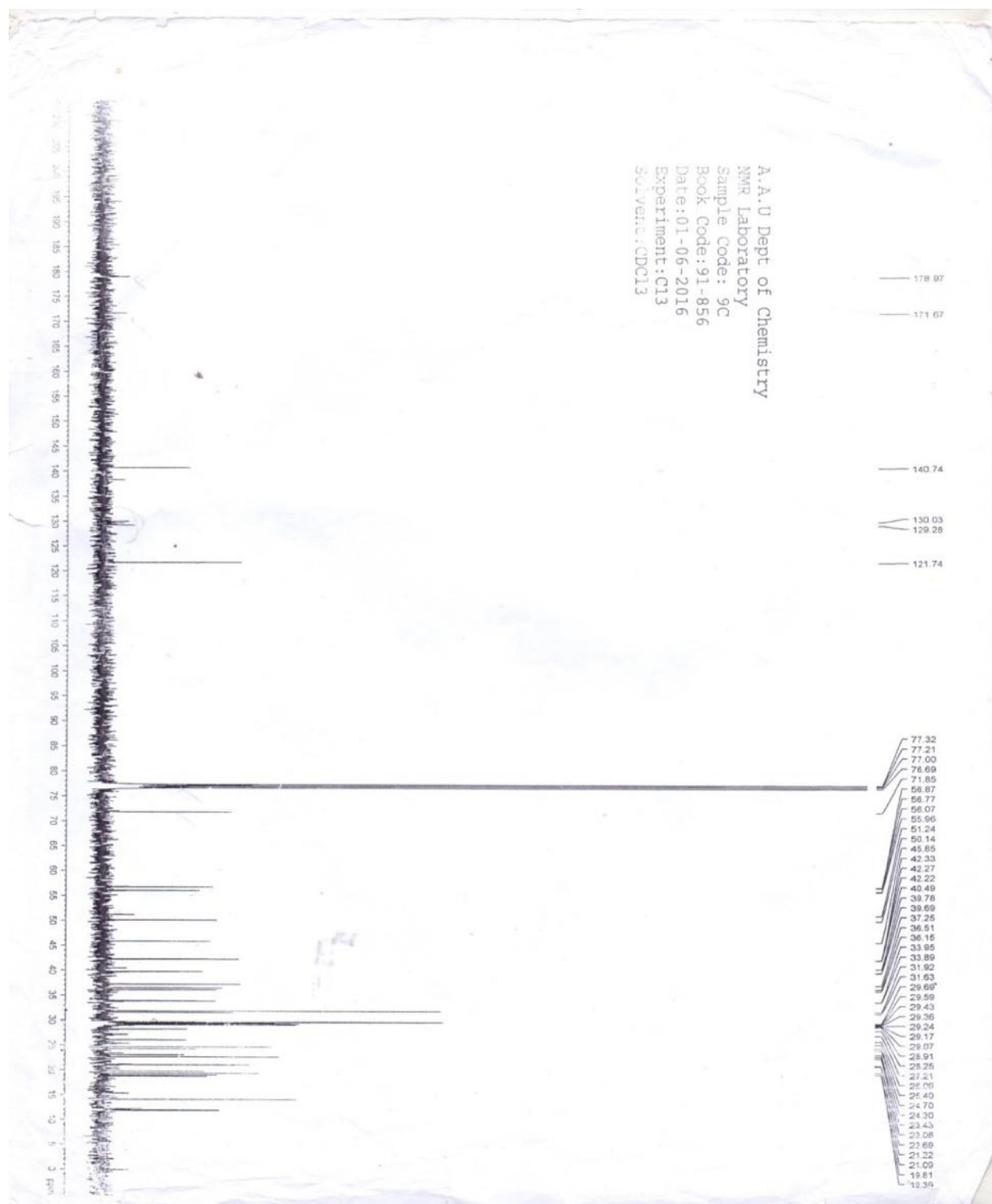
32. Jaradat, N., Hussen F., Ali A. A., (2015). Preliminary Phytochemical Screening, quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of *Ephedra alata* Decene. *J. Mater Environ. Sci* 6(6) (2015) 1717-1778 ISSN:2028-2508 CODEN:JMESC�, Palestine P.O.Box 7.
33. Bernfiel (1963). Biogenesis of Natural products p617-19, New, York
MACMILLAN 24. Mann. J. (1995) Secondary Metabolites p279, New York, Oxford University Press
34. <http://www.theplantlist.org/tpl/record/kew-2766480>, Nov, 2015 Addis Ababa
35. Bhatt, Z. A., Naidoo, Y., and Nicolas, A., (2010) *Biol. Res.* 43:403-409,
36. Kinjal, S M., Dipal, T., & Devange R. K. P., (2013), Quality control parameters to detect adulteration of market samples of Indian Manjstha by Iranian Manjstha. College of pharmacy, Tramba Rai, Kot-20, Gujarat, India. E-mail Pandadevenge82@yahoo.com Received 06, 12, 2012, revised 13, 12, 2013
37. [http://www.kibs.co.ke/eaherbs.php?herb=Dicliptera% 20 laxata](http://www.kibs.co.ke/eaherbs.php?herb=Dicliptera%20laxata), Nov. 2015. Addis Ababa
38. Luo, Y., Feng, C., Tian, Y., Zhang, G., (2002). Glycosides from *Dicliptera riparia*. *Phytochemistry*; 61:449-54.
39. Li, X., Liu, Z., Chen, Y., Wang, L. J., Zeng, Y. N., Sun, G. Z., and Ruan, C. C., (2009) Rubiacordone A: A new Anthraquinone Glycoside, from the Roots of *Rubia cordifolia*, WWW.mdpi.com/Journal/Molecules, 14, 566-572.
40. Lulekal, E., Asfaw, Z., Kelbesa, E and Van, D. P., (2014). *Journal of Ethnobiology and Ethnomedicine*, 10:21
41. Ngussie, G., (1999). phytochemical investigation on leaves of *Bulbine abyssinica*, *Bulbin natalensis*, *Trachyy* and *radivaricata*, AAU 7.
42. Woldemariam, M., (2005). phytochemical investigation on leaves of *Senna sophera* p.36, AAU
43. [http://www.tropics.org/Name/103465\(GroupDicot\)](http://www.tropics.org/Name/103465(GroupDicot)) ... Missouri Botanical Garden 4344 Shau Boulevard saint Louis Missouri 63110, Nov. 2015 Addis Ababa.
44. [http://www.icp.online.can/documents/file///D:/sterol/vol II Issue 9/03 pdf](http://www.icp.online.can/documents/file///D:/sterol/vol%20II%20Issue%209/03.pdf), Aug, 2016, Adama
45. Parvin, S., Abdulkader, Md., Abdul, Muhit, Md., Ekramal H, Md and Imam, Ibune, W, M., (2011). Triterpenoids and phytosteroids from stem bark of *Crataeva nurulabuchham*, *Journal of Applied Pharmaceutical Science* 01(09); 2011:47-50, Univ of Dhaka, Dhaka Bangladesh
46. Pierre, L. L., Moses, M. N., (2015). Isolation and Characterization of Stigmasterol and Sitosterol from *Odotonema strictum* (*Acanthaceae*), *JIPBS*, vol 2(1), 88-95, Lusaka, Zambia)

47. *Journal of ethnopharmacology genus Stephania (Menispermaceae), chemical and pharmacological perspectives*. Available online 27 Aug 2010 (s 14213e.pdf.)
48. Sandrove, N., and Jones, G., (2015). A contemporary Introduction to Essential oils: Chemistry, Bioactivity and Prospects for Australian Agriculture. Australia, E-mail: nsadgro@une.edu.au published 3 March.
49. Wikipedia, *β - Sitosterol*, sept. 2016. Addis Ababa.

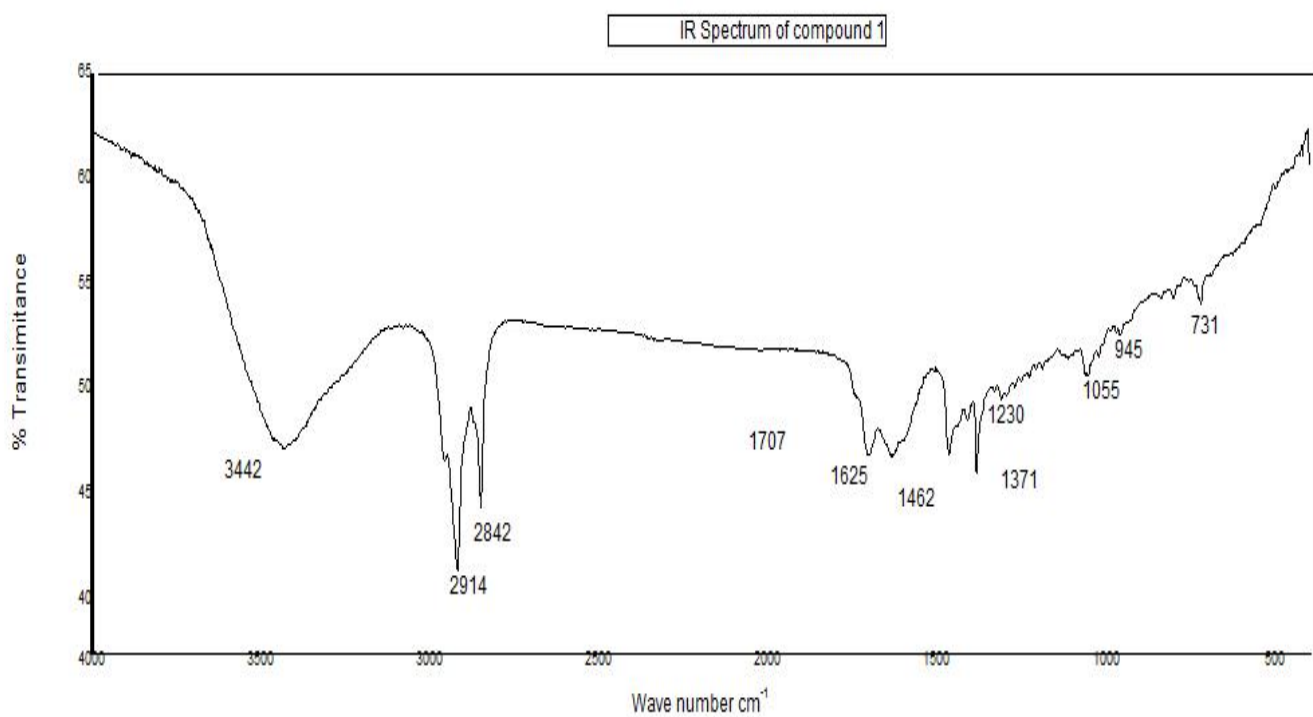
APPENDICES



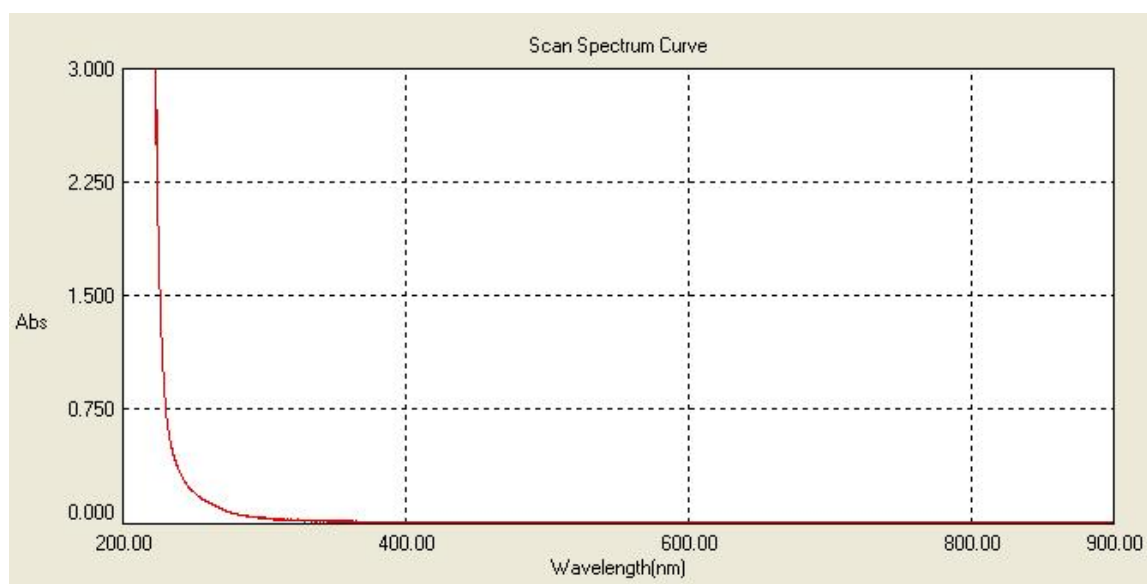
Appendix 1. ¹H NMR spectrum of compound 1



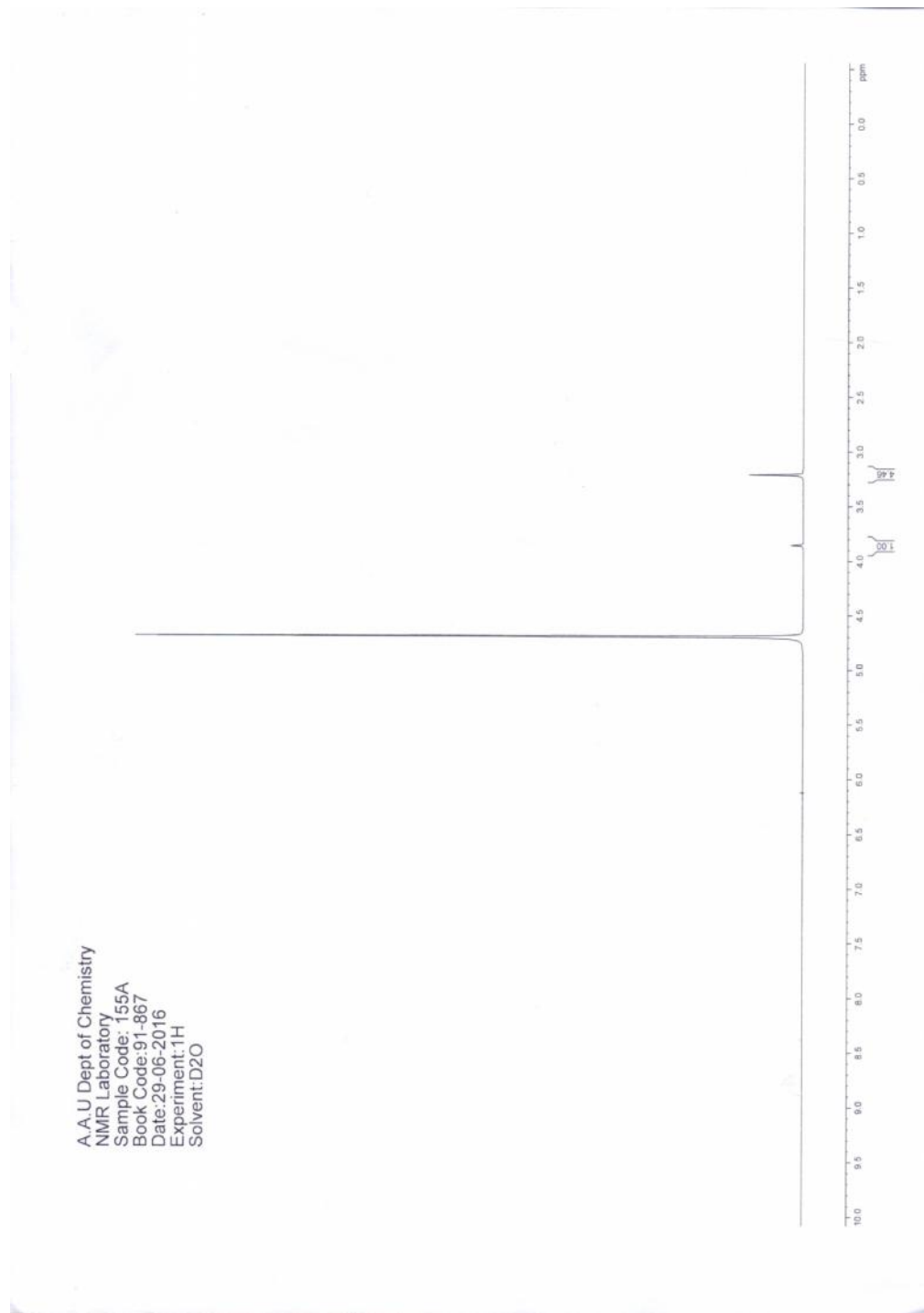
Appendix 2. ^{13}C NMR spectrum of compound **1**



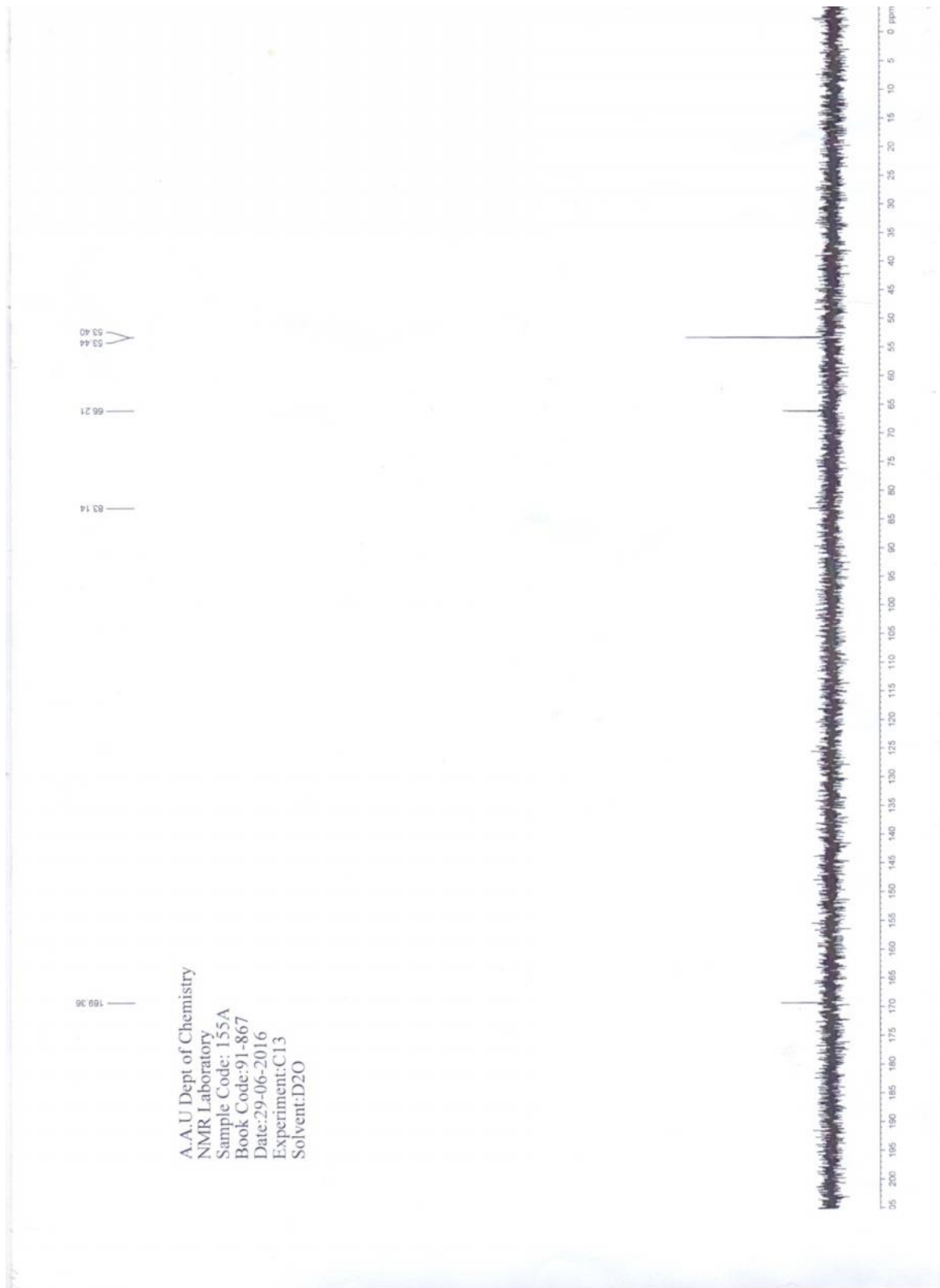
Appendix 4. IR spectrum of compound 1



Appendix 5. UV spectrum of compound 1



Appendix 6. ¹H NMR spectrum of compound **2**

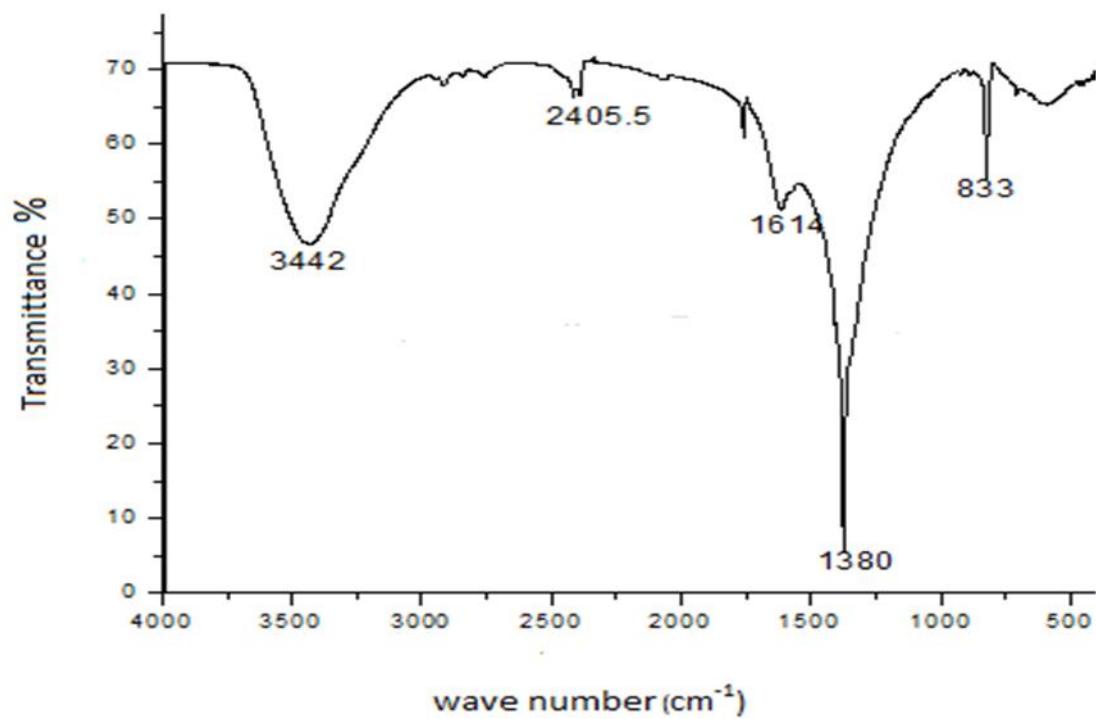


Appendix 7. ^{13}C NMR spectrum of compound 2

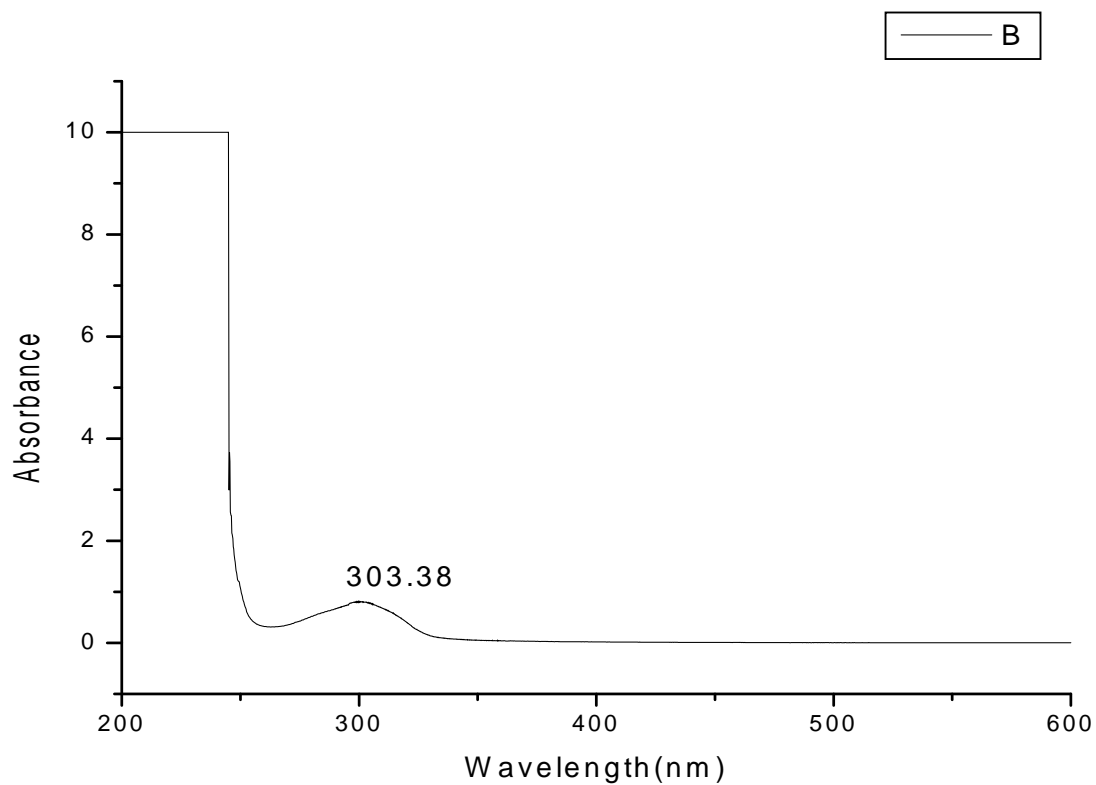
A.A.U Dept of Chemistry
NMR Laboratory
Sample Code: 155A
Book Code: 91-867
Date: 29-06-2016
Experiment: Dept-135
Solvent: D2O

300 195 180 165 150 135 120 115 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

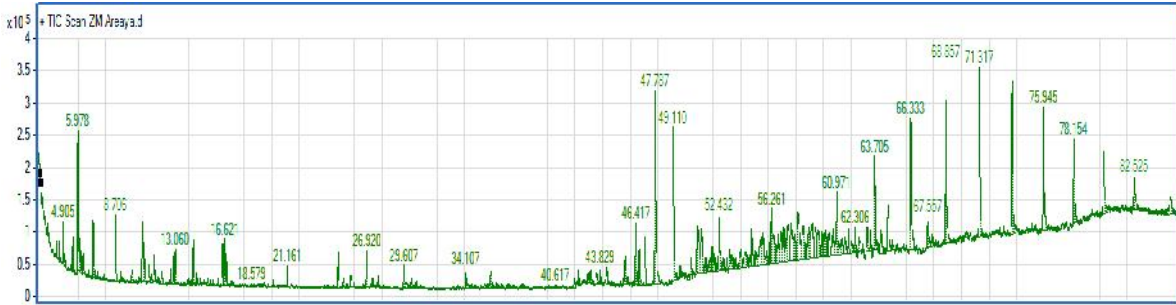
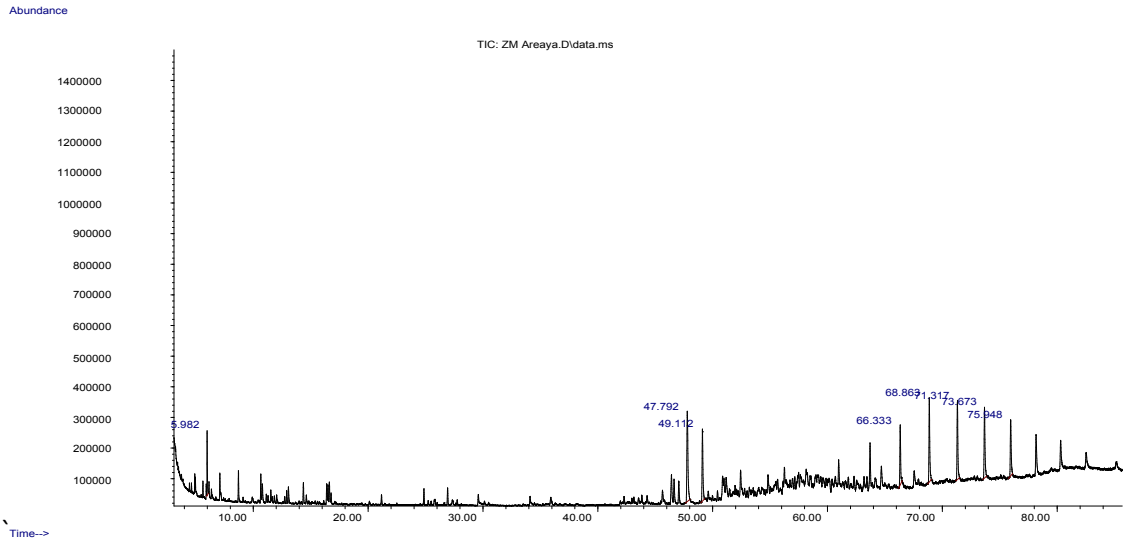
Appendix 8. DEPT spectrum of compound 2



Appendix 9. IR spectrum of compound 2



Appendix 10. UV spectrum of compound 2



Appendix .11 ZM

Area Percent Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : ZM Areaya.D

Acq On : 16 May 2016 01:36

Operator : Teshome

Sample : ZM Areaya

Misc :

ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Training.M

Title :

Signal : TIC: ZM Areaya.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.982	492	497	515	VB	208540	5211631	26.54%	5.486%
2	47.792	7645	7670	7710	BB 3	295296	19639072	100.00%	20.673%
3	49.112	7874	7896	7928	BB 2	233863	12150377	61.87%	12.790%
4	66.333	10830	10851	10878	BB 2	195978	9136842	46.52%	9.618%
5	68.863	11264	11285	11317	BB 4	274777	13342426	67.94%	14.045%
6	71.317	11684	11706	11736	BB 2	256895	13105968	66.73%	13.796%
7	73.673	12086	12110	12142	BB 3	230316	12459918	63.44%	13.116%
8	75.948	12467	12501	12528	BB 4	182605	9950659	50.67%	10.475%

Sum of corrected areas: 94996892

Training.M Mon May 16 03:39:23 2016

Library Search Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : ZM Areaya.D

Acq On : 16 May 2016 01:36

Operator : Teshome

Sample : ZM Areaya

Misc :

ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 90

D:\MassHunter\Library\demo.l Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
-----	----	-------	------------	------	------	------

1	5.982	5.49	D:\MassHunter\Library\demo.l			
---	-------	------	------------------------------	--	--	--

No matches found

2	47.792	20.67	D:\MassHunter\Library\demo.l			
---	--------	-------	------------------------------	--	--	--

No matches found

3	49.112	12.79	D:\MassHunter\Library\NIST11.L			
---	--------	-------	--------------------------------	--	--	--

			Benzene, (1-methyldodecyl)-	111020	004534-53-6	95
--	--	--	-----------------------------	--------	-------------	----

			Benzene, (1-methyldodecyl)-	111019	004534-53-6	74
--	--	--	-----------------------------	--------	-------------	----

			Benzene, (1-methyldecyl)-	87788	004536-88-3	64
--	--	--	---------------------------	-------	-------------	----

4	66.333	9.62	D:\MassHunter\Library\NIST11.L			
---	--------	------	--------------------------------	--	--	--

Hexacosane			194493	000630-01-3		94
------------	--	--	--------	-------------	--	----

Heptadecane			94346	000629-78-7		93
-------------	--	--	-------	-------------	--	----

Tetracosane			175556	000646-31-1		91
-------------	--	--	--------	-------------	--	----

5 68.863 14.05 D:\MassHunter\Library\NIST11.L

Tetracosane 175558 000646-31-1 97

Eicosane 129490 000112-95-8 95

2-methyloctacosane 215181 1000376-72-8 90

6 71.317 13.80 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 95

Hexadecane 83023 000544-76-3 95

Eicosane 129492 000112-95-8 94

7 73.673 13.12 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 95

Octadecane 105885 000593-45-3 93

Tetratetracontane 241528 007098-22-8 93

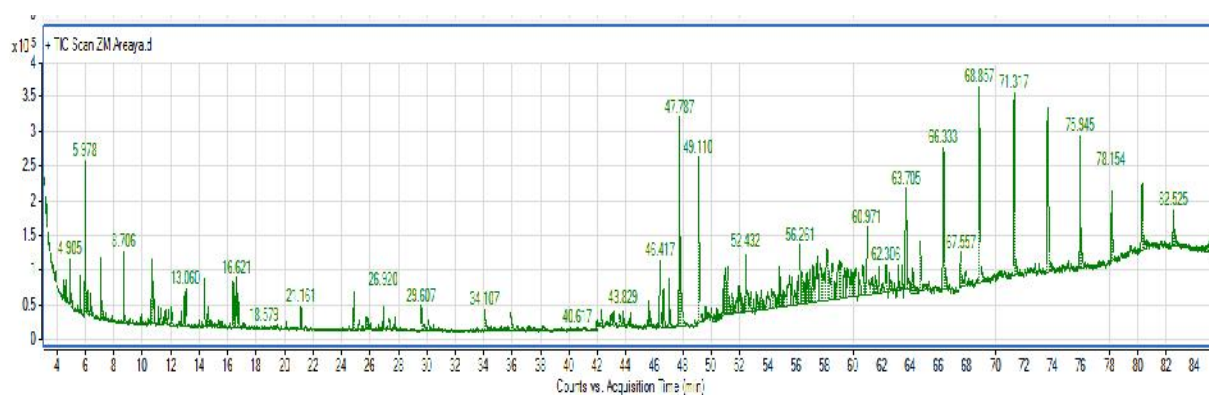
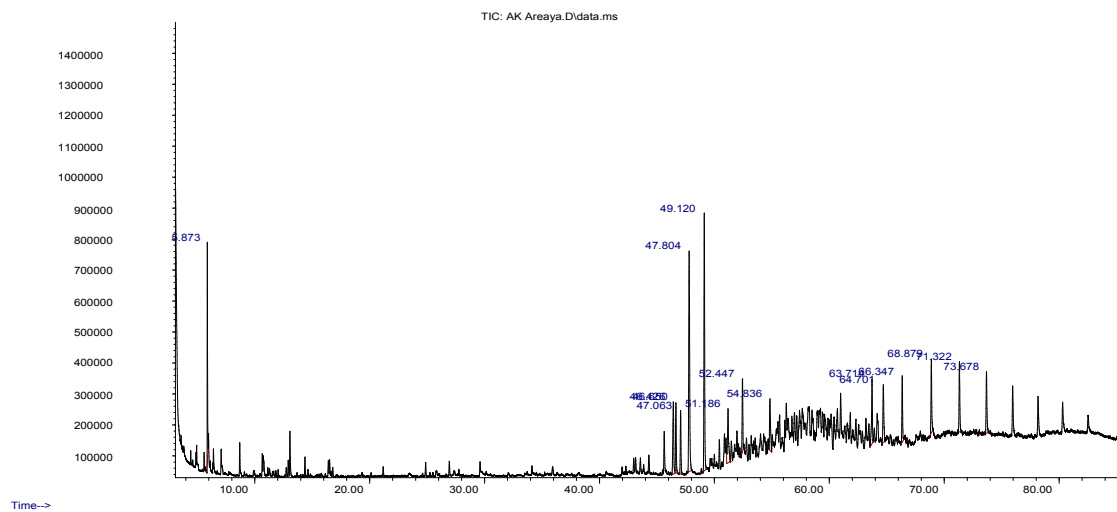
8 75.948 10.47 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 95

Octadecane 105885 000593-45-3 92

Eicosane 129492 000112-95-8 90

Training.M Mon May 16 03:37:08 2016



Area Percent Report

Appendix .12 AK

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : AK Areaya.D

Acq On : 15 May 2016 23:57

Operator : Teshome

Sample : AK Areaya

Misc :

ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Training.M

Title :

Signal : TIC: AK Areaya.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max	% of total
1	5.873	465	478	492	BV	711246	15253341	37.28%	6.990%
2	46.426	7413	7436	7458	BV 2	232581	13090629	31.99%	5.999%
3	46.650	7458	7474	7495	VB	225927	9683425	23.67%	4.437%
4	47.063	7522	7545	7570	BB 3	205244	9040621	22.10%	4.143%
5	47.804	7645	7672	7714	BB 3	711129	40915375	100.00%	18.749%
6	49.120	7868	7898	7931	BB	826159	37222791	90.98%	17.057%
7	51.186	8232	8252	8286	VB 2	174592	12231151	29.89%	5.605%
8	52.447	8443	8469	8482	BV	234495	9986519	24.41%	4.576%
9	54.836	8864	8878	8903	PV 9	163796	9506989	23.24%	4.357%
10	63.714	10371	10402	10418	PV 6	207287	11018470	26.93%	5.049%
11	64.701	10545	10571	10601	BV	186503	10268471	25.10%	4.706%
12	66.347	10830	10853	10877	BB 2	212532	9264746	22.64%	4.246%
13	68.879	11253	11288	11313	BV 3	238095	10563651	25.82%	4.841%
14	71.322	11687	11707	11734	BV 4	227309	10417026	25.46%	4.774%
15	73.678	12086	12111	12140	BB 3	198666	9759025	23.85%	4.472%

Sum of corrected areas: 218222229

Training.M Mon May 16 03:38:10 2016

Library Search Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : AK Areaya.D

Acq On : 15 May 2016 23:57

Operator : Teshome

Sample : AK Areaya

Misc :

ALS Vial : 1 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 90

D:\MassHunter\Library\demo.l Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
-----	----	-------	------------	------	------	------

1	5.873	6.99	D:\MassHunter\Library\NIST11.L			
			2-Hexenal, (E)-	3174	006728-26-3	98
			2-Hexenal, (E)-	3171	006728-26-3	97
			2-Hexenal, (E)-	3172	006728-26-3	96

2	46.426	6.00	D:\MassHunter\Library\demo.l			
---	--------	------	------------------------------	--	--	--

Library Search Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\
 Data File : AK Areaya.D
 Acq On : 15 May 2016 23:57
 Operator : Teshome
 Sample : AK Areaya
 Misc :
 ALS Vial : 1 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 90
 D:\MassHunter\Library\W9N11.L Minimum Quality: 0

Unknown Spectrum: Apex
 Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.873	6.99	D:\MassHunter\Library\NIST11.L			
			2-Hexenal, (E)-	3174	006728-26-3	98
			2-Hexenal, (E)-	3171	006728-26-3	97
			2-Hexenal, (E)-	3172	006728-26-3	96
2	46.426	6.00	D:\MassHunter\Library\W9N11.L			
			Benzene, (1-pentylloctyl)- (CAS) \$	353877	004534-49-0	89
			Tridecane, 6-phenyl-			
			Benzene, (1-pentylloctyl)- \$	353876	004534-49-0	89
			cane, 6-phenyl- \$ (1-Pentylloctyl)			
			benzene #			
			Benzene, (1-hexylheptyl)- \$	353883	002400-01-3	62
			cane, 7-phenyl- \$ 7-Phenyltrideca			
			ne			
3	46.650	4.44	D:\MassHunter\Library\W9N11.L			
			Benzene, (1-butylonyl)- \$	353878	004534-50-3	64
			ane, 5-phenyl- \$ (1-Butylonyl)be			
			nzene #			
			Benzene, (1-butylloctyl)- \$	313713	002719-63-3	43
			ne, 5-phenyl- \$ 5-Phenylododecane			
			Benzene, (1,1-diethylpropyl)- \$ (122762	004170-84-7	43
			1,1-Diethylpropyl)benzene			
4	47.063	4.14	D:\MassHunter\Library\W9N11.L			
			Benzene, (1-propyldecyl)- \$	353879	004534-51-4	64
			cane, 4-phenyl- \$ (1-Propyldecyl)			
			benzene #			
			4-phenylnonane \$	193961	065185-83-3	59
			ylhexyl)- (CAS)			
			Benzene, (1-propylonyl)- (CAS) \$	313708	002719-64-4	52
			4-Phenylododecane \$			
			Dodecane, 4-p			
			henyl-			
5	47.804	18.75	D:\MassHunter\Library\W9N11.L			
			1,2-Benzenedicarboxylic acid, bis(403889	000084-69-5	72
			2-methylpropyl) ester (CAS)			
			Phthalic acid, 4-cyanophenyl hepty	617531	998617-53-1	72
			l ester			
			Phthalic acid, isobutyl phenyl est	460219	998460-21-9	64
			er			
6	49.120	17.06	D:\MassHunter\Library\NIST11.L			
			Benzene, (1-methyldodecyl)-	111020	004534-53-6	94
			Benzene, (1-methyldodecyl)-	111019	004534-53-6	87
			Benzene, (1-methyldecyl)-	87787	004536-88-3	72
7	51.186	5.60	D:\MassHunter\Library\W9N11.L			
			Benzene, (1-ethylododecyl)- (CAS) \$	394346	004534-58-1	53
			\$ 3-Phenyltetradecane			

Training.M Sat May 21 01:11:22 2016

Pa 1

10 63.714 5.05 D:\MassHunter\Library\NIST11.L

Tetracosane 175557 000646-31-1 98

Tetracosane 175559 000646-31-1 98

Tetracosane 175558 000646-31-1 93

11 64.701 4.71 D:\MassHunter\Library\demo.l

No matches found

12 66.347 4.25 D:\MassHunter\Library\NIST11.L

Tricosane 164578 000638-67-5 97

Tetracosane 175557 000646-31-1 96

Eicosane 129493 000112-95-8 95

13 68.879 4.84 D:\MassHunter\Library\NIST11.L

Octadecane 105885 000593-45-3 93

Tetratetracontane 241528 007098-22-8 87

Heptacosane, 1-chloro- 217413 062016-79-9 87

14 71.322 4.77 D:\MassHunter\Library\NIST11.L

Tetracosane 175559 000646-31-1 96

Heneicosane 141426 000629-94-7 92

Nonadecane 117638 000629-92-5 90

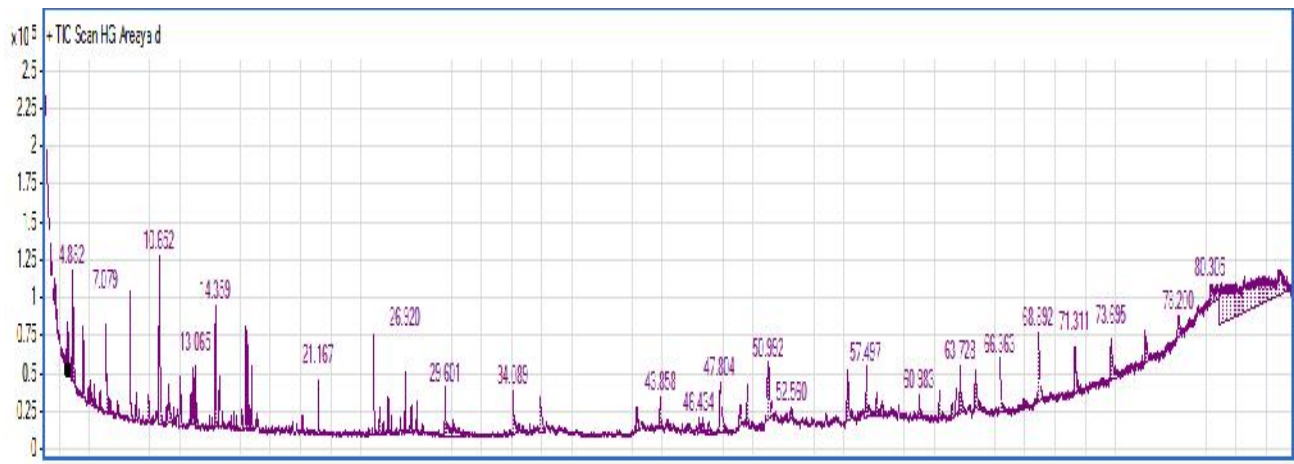
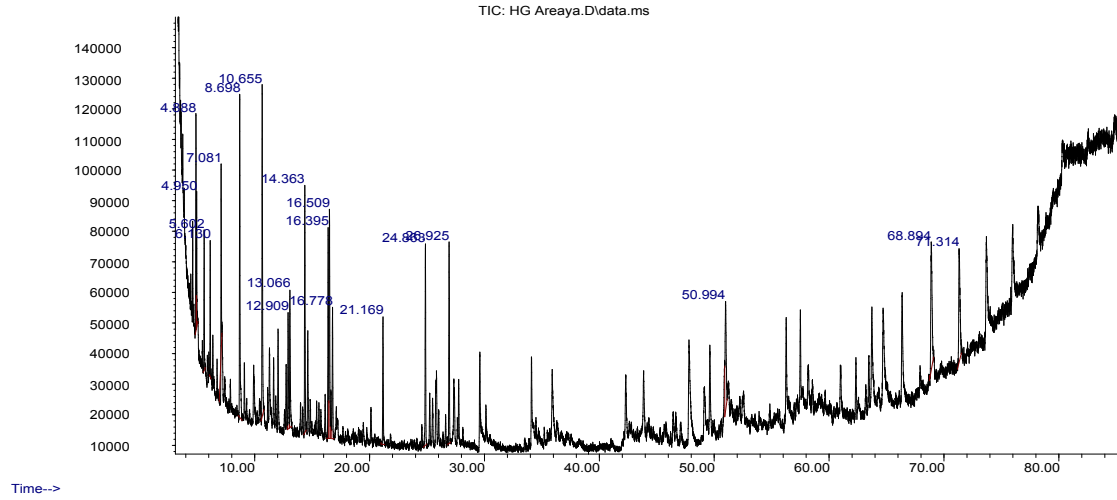
15 73.678 4.47 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 95

Octadecane 105885 000593-45-3 91

Eicosane 129492 000112-95-8 91

Abundance



Appendix 13 HG

Area Percent Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : HG Areaya.D

Acq On : 16 May 2016 03:16

Operator : Teshome

Sample : HG Areaya

Misc :

ALS Vial : 3 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Training.M

Title :

Signal : TIC: HG Areaya.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max	% of total
1	4.888	298	309	315	PV 2	71214	1446259	38.37%	3.563%
2	4.950	315	320	337	VV	43896	1100505	29.19%	2.711%
3	5.602	419	431	445	PB 5	44961	1107661	29.38%	2.729%
4	6.130	511	522	532	BV 4	44325	1082731	28.72%	2.667%
5	7.081	658	685	695	BV	61622	1042017	27.64%	2.567%
6	8.698	941	963	996	BB 2	105191	3752954	99.56%	9.245%
7	10.655	1281	1298	1322	BV 3	107994	3769549	100.00%	9.286%
8	12.909	1675	1685	1703	BV 8	38065	1387599	36.81%	3.418%
9	13.066	1703	1712	1734	VB 9	43776	1746674	46.34%	4.303%
10	14.363	1918	1935	1953	BV 4	79805	2823391	74.90%	6.955%
11	16.395	2265	2283	2293	BV 3	68380	2467263	65.45%	6.078%
12	16.509	2293	2303	2317	VV 4	74743	2826330	74.98%	6.962%
13	16.778	2337	2349	2368	VB 2	42546	1545003	40.99%	3.806%
14	21.169	3073	3102	3124	BV 4	41226	1455125	38.60%	3.585%

15 24.863 3707 3736 3752 BV 3 65540 2336363 61.98% 5.755%
16 26.925 4070 4090 4107 PV 3 65276 2364314 62.72% 5.824%
17 50.994 8206 8219 8242 VV 9 35556 2435781 64.62% 6.000%
18 68.894 11263 11290 11322 BV 6 41937 3086420 81.88% 7.603%
19 71.314 11680 11706 11741 PV 6 36769 2817974 74.76% 6.942%

Sum of corrected areas: 40593913

Training.M Mon May 16 06:58:57 2016

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : HG Areaya.D

Acq On : 16 May 2016 03:16

Operator : Teshome

Sample : HG Areaya

Misc :

ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 90

D:\MassHunter\Library\demo.l Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
-----	----	-------	------------	------	------	------

1	4.888	3.43	D:\MassHunter\Library\demo.l			
---	-------	------	------------------------------	--	--	--

No matches found

2 4.950 2.61 D:\MassHunter\Library\demo.l

No matches found

3 5.602 2.63 D:\MassHunter\Library\demo.l

No matches found

4 6.130 2.57 D:\MassHunter\Library\demo.l

No matches found

5 7.081 2.47 D:\MassHunter\Library\NIST11.L

Styrene 4873 000100-42-5 96

Bicyclo[4.2.0]octa-1,3,5-triene 4882 000694-87-1 94

Styrene 4875 000100-42-5 91

6 8.698 8.81 D:\MassHunter\Library\NIST11.L

.alpha.-Pinene 15705 000080-56-8 96

(1R)-2,6,6-Trimethylbicyclo[3.1.1] 15854 007785-70-8 94

hept-2-ene

.alpha.-Pinene 15704 000080-56-8 91

7 10.655 8.94 D:\MassHunter\Library\demo.l

No matches found

8 12.909 3.29 D:\MassHunter\Library\demo.l

No matches found

9 13.066 4.14 D:\MassHunter\Library\NIST11.L

Eucalyptol 26615 000470-82-6 91
Eucalyptol 26625 000470-82-6 53
Trifluoroacetyl-.alpha.-terpineol 101946 1000058-17-6 45

10 14.363 6.69 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 12

11 16.395 5.86 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 40

12 16.509 6.72 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 50

13 16.778 3.75 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 9

14 21.169 3.45 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 70

15 24.863 5.54 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 72

16 26.925 5.61 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 50

17 50.994 5.78 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 7

18 56.267 3.73 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 4

19 68.894 7.32 D:\MassHunter\Library\demo.l

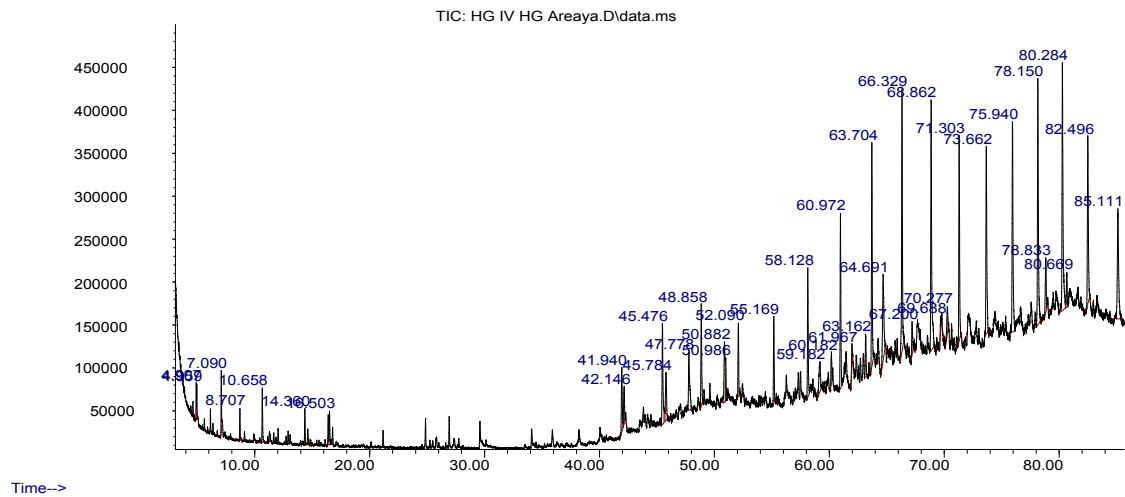
Dodecane 1 000112-40-3 14

20 71.314 6.68 D:\MassHunter\Library\demo.l

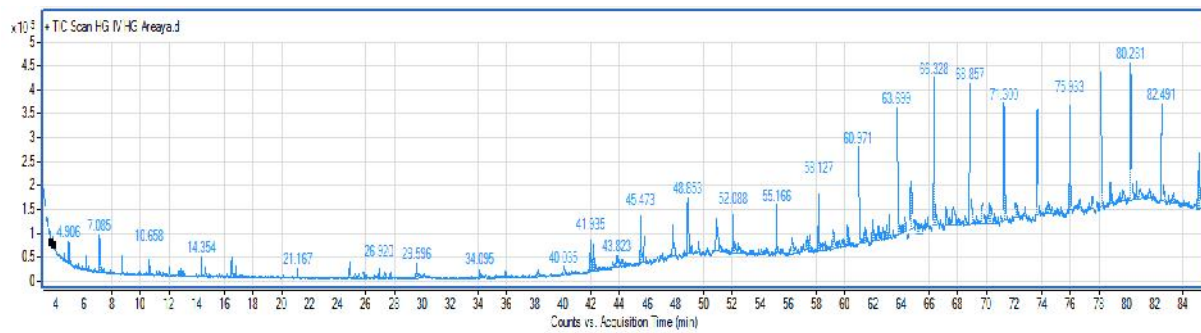
Dodecane 1 000112-40-3 25

Training.M Mon May 16 07:00:00 2016

Abundance



Time-->



Appendix 14 Area Percent Report HG IV

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : HG IV HG Areaya.D

Acq On : 16 May 2016 04:56

Operator : Teshome

Sample : HG IV HG Areaya

Misc :

ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Training.M

Title :

Signal : TIC: HG IV HG Areaya.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max	% of total
1	4.907	295	312	317	PV 3	42724	879127	5.09%	0.389%
2	4.959	317	321	335	VB	39610	852023	4.93%	0.377%
3	7.090	674	687	700	PV 2	69828	1817498	10.52%	0.804%
4	8.707	947	964	985	BB 4	36977	1158611	6.70%	0.512%
5	10.658	1282	1299	1321	BB 5	63108	2197727	12.72%	0.972%
6	14.360	1917	1934	1950	BV 6	42326	1483699	8.59%	0.656%
7	16.503	2293	2302	2317	VV 3	38464	1320238	7.64%	0.584%
8	41.940	6645	6666	6686	PV 4	74985	3950951	22.86%	1.747%
9	42.146	6686	6701	6717	VV 6	43499	2370198	13.71%	1.048%
10	45.476	7254	7273	7295	PV 4	117199	6359462	36.80%	2.812%
11	45.784	7310	7325	7347	VV 7	55736	3622873	20.96%	1.602%

12 47.778 7653 7667 7705 BV 2 67287 4973573 28.78% 2.199%
13 48.858 7838 7853 7872 BV 2 118652 5426483 31.40% 2.400%
14 50.882 8180 8200 8212 VV 69887 4415946 25.55% 1.953%
15 50.986 8212 8218 8236 VV 8 49592 2197942 12.72% 0.972%

16 52.090 8391 8407 8423 BV 4 85157 3487405 20.18% 1.542%
17 55.169 8922 8936 8953 BV 3 100708 4316077 24.97% 1.909%
18 58.128 9424 9443 9467 PV 2 149936 6798148 39.34% 3.006%
19 59.182 9579 9624 9645 PV 2 34283 3335643 19.30% 1.475%
20 60.182 9770 9796 9807 PV 8 36926 1573249 9.10% 0.696%

21 60.972 9913 9931 9954 PV 202377 8939897 51.73% 3.953%
22 61.967 10083 10102 10110 PV 9 27215 907816 5.25% 0.401%
23 63.162 10288 10307 10323 PV 9 49773 2474310 14.32% 1.094%
24 63.704 10378 10400 10427 PV 2 260322 12244508 70.85% 5.415%
25 64.691 10530 10569 10599 PV 7 99584 8151300 47.17% 3.605%

26 66.329 10819 10850 10880 PV 3 304386 15469280 89.51% 6.841%
27 67.200 10981 11000 11017 BV 3 38269 1816413 10.51% 0.803%
28 68.862 11261 11285 11329 BV 2 290884 16042974 92.83% 7.095%
29 69.688 11406 11427 11434 PV 8 16627 398200 2.30% 0.176%
30 70.277 11493 11528 11540 BV 8 31185 1931096 11.17% 0.854%

31 71.303 11685 11704 11725 BV 3 237868 10772293 62.33% 4.764%
32 73.662 12092 12108 12136 PV 2 217151 11682689 67.60% 5.166%
33 75.940 12483 12499 12533 VV 2 244284 13469352 77.94% 5.956%
34 78.150 12855 12878 12915 PV 3 285230 15836301 91.64% 7.003%
35 78.833 12978 12995 13013 PV 3 62444 3442764 19.92% 1.522%

36 80.284 13222 13244 13287 VV 2 288236 17281843 100.00% 7.642%
37 80.669 13294 13311 13326 VV 2 39532 1966928 11.38% 0.870%
38 82.496 13592 13624 13649 BV 5 195041 10991328 63.60% 4.861%
39 85.111 14051 14073 14115 VV 6 126554 9776113 56.57% 4.323%

Sum of corrected areas: 226132280

Training.M Mon May 16 07:01:36 2016

Library Search Report

Data Path : D:\MassHunter\Data\Essential oil\Yosef oil\

Data File : HG IV HG Areaya.D

Acq On : 16 May 2016 04:56

Operator : Teshome

Sample : HG IV HG Areaya

Misc :

ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 90

D:\MassHunter\Library\demo.l Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
-----	----	-------	------------	------	------	------

1	4.907	0.39	D:\MassHunter\Library\demo.l			
---	-------	------	------------------------------	--	--	--

No matches found

2 4.959 0.38 D:\MassHunter\Library\demo.l

No matches found

3 7.090 0.80 D:\MassHunter\Library\NIST11.L

Styrene 4873 000100-42-5 96

Bicyclo[4.2.0]octa-1,3,5-triene 4882 000694-87-1 96

1,3,5,7-Cyclooctatetraene 4880 000629-20-9 94

4 8.707 0.51 D:\MassHunter\Library\NIST11.L

(1R)-2,6,6-Trimethylbicyclo[3.1.1] 15852 007785-70-8 94

hept-2-ene

.alpha.-Pinene 15705 000080-56-8 90

Bicyclo[3.1.1]heptane, 6,6-dimethy 15908 018172-67-3 87

l-2-methylene-, (1S)-

5 10.658 0.97 D:\MassHunter\Library\demo.l

No matches found

6 14.360 0.66 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 33

7 16.503 0.58 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 28

8 41.940 1.75 D:\MassHunter\Library\NIST11.L

Heptadecane 94345 000629-78-7 96

Heptadecane 94346 000629-78-7 95

Octacosane 209566 000630-02-4 81

9 42.146 1.05 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 64

10 45.476 2.81 D:\MassHunter\Library\NIST11.L

Octadecane 105885 000593-45-3 94

Hexacosane 194493 000630-01-3 91

Nonadecane 117637 000629-92-5 91

11 45.784 1.60 D:\MassHunter\Library\NIST11.L

Sulfurous acid, butyl tetradecyl e 172308 1000309-18-1 91

ster

Tetratetracontane 241528 007098-22-8 91

Dodecane, 2-methyl- 48854 001560-97-0 89

12 47.778 2.20 D:\MassHunter\Library\demo.l

No matches found

13 48.858 2.40 D:\MassHunter\Library\NIST11.L

Nonadecane 117637 000629-92-5 96

Nonadecane 117638 000629-92-5 93

Decane, 3,8-dimethyl- 38349 017312-55-9 91

14 50.882 1.95 D:\MassHunter\Library\demo.l

No matches found

15 50.986 0.97 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 9

16 52.090 1.54 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 95

Eicosane 129492 000112-95-8 91

Hexacosane 194493 000630-01-3 87

17 55.169 1.91 D:\MassHunter\Library\NIST11.L

Heneicosane 141425 000629-94-7 97

Heneicosane 141426 000629-94-7 96

Eicosane 129490 000112-95-8 96

18 58.128 3.01 D:\MassHunter\Library\NIST11.L

Heneicosane 141426 000629-94-7 95

Hexacosane 194493 000630-01-3 91

Tetratetracontane 241528 007098-22-8 91

19 59.182 1.48 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 16

20 60.182 0.70 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 10

21 60.972 3.95 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 97

Tetratetracontane 241528 007098-22-8 91

Hexacosane 194493 000630-01-3 91

22 61.967 0.40 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 10

23 63.162 1.09 D:\MassHunter\Library\demo.l

No matches found

24 63.704 5.41 D:\MassHunter\Library\NIST11.L

Tetracosane 175556 000646-31-1 96

Tetracosane 175559 000646-31-1 96

Tetracosane 175557 000646-31-1 96

25 64.691 3.60 D:\MassHunter\Library\demo.l

No matches found

26 66.329 6.84 D:\MassHunter\Library\NIST11.L

Eicosane 129493 000112-95-8 94

Tetratetracontane 241528 007098-22-8 91

2-methyloctacosane 215181 1000376-72-8 91

27 67.200 0.80 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 27

28 68.862 7.09 D:\MassHunter\Library\NIST11.L

Hexacosane 194492 000630-01-3 96

Octadecane 105885 000593-45-3 93

Tritetracontane 241174 007098-21-7 91

29 69.688 0.18 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 93

Sulfurous acid, octadecyl 2-propyl 200449 1000309-12-7 76

ester

Eicosane 129491 000112-95-8 72

30 70.277 0.85 D:\MassHunter\Library\demo.l

Dodecane 1 000112-40-3 10

31 71.303 4.76 D:\MassHunter\Library\NIST11.L

Hexadecane, 1-iodo- 184946 000544-77-4 92

Heptacosane, 1-chloro- 217413 062016-79-9 91

Tritetracontane 241174 007098-21-7 91

32 73.662 5.17 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 93

Pentadecane 71394 000629-62-9 93

Eicosane 129492 000112-95-8 92

33 75.940 5.96 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 93

Octadecane 105885 000593-45-3 92

3-Eicosene, (E)- 127771 074685-33-9 92

34 78.150 7.00 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 98

Nonadecane 117638 000629-92-5 91

Eicosane 129492 000112-95-8 91

35 78.833 1.52 D:\MassHunter\Library\demo.l

No matches found

36 80.284 7.64 D:\MassHunter\Library\NIST11.L

Eicosane 129490 000112-95-8 98

Octadecane 105885 000593-45-3 95

Pentadecane 71394 000629-62-9 90

37 80.669 0.87 D:\MassHunter\Library\demo.l

No matches found

38 82.496 4.86 D:\MassHunter\Library\NIST11.L

Octadecane 105885 000593-45-3 91

Heptadecane 94344 000629-78-7 90

Nonadecane 117638 000629-92-5 90

39 85.111 4.32 D:\MassHunter\Library\NIST11.L

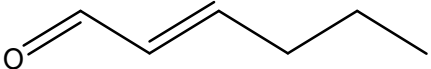
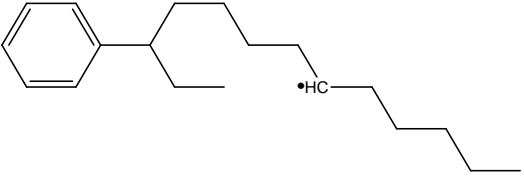
Octadecane 105885 000593-45-3 93

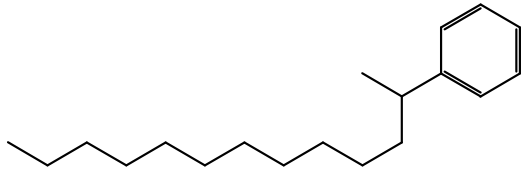
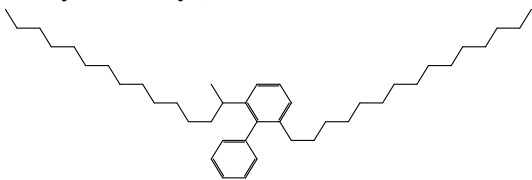
Octadecane 105886 000593-45-3 87

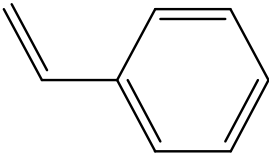
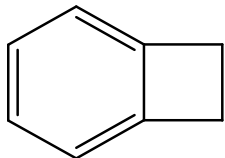
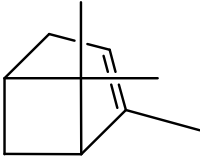
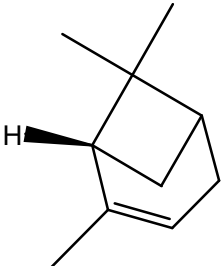
Eicosane 129491 000112-95-8 86

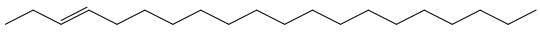
Training.M Mon May 16 07:01:56 2016

Appendix Table 1. The essential oils composition of *D. laxata* with 70 % qualitative availability

No	Structure and name of the compound	Sample ZM % ^{PK} ,...	Sample AK % ^{PK} ,...	Sample HG % ^{PK} ,...	Sample H G IV % ^{PK} ,...	Total n ^o frequency of library records
1	 2-Hexenal,(E)-		98 ¹ ,87 ¹ ,96 ¹			3
2	Benzene,(1-pentyl)octyl- (CAS)Tridacane,6-phenyl 		89 ²			1
3	Benzene,(1-pentyl)octyl-Tridacane,6-phenyl-(1-pentyl)benzene		89			
4	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester(CAS		72 ¹			1
5	Phthalic acid,4-cynophenyl heptyl ester		72 ¹			1
6	2-methyloctacosane	90 ⁵			91 ²⁶	2

7	Benezene,(1-methyldecyl) 	95 ³ ,74 ³	94 ⁶ ,87 ⁶		
7	Bezene,(1-methyldecyl)		72 ⁶		
8	Bezene,(1-methylpentadecyl)Hexadecane,2-phenyl-		90 ⁸		
9	Pentadecane,2-phenyl-(1-methyltetradecyl)benzene 		70 ⁸		
10	Hexacosane:CH ₃ -(CH ₂) ₂₄ -CH ₃	94 ⁴			
11	Heptadecane:CH ₃ -(CH ₂) ₁₅ -CH ₃	93 ⁴			98 ³⁸
12	Longifolene.		86 ⁹		
13	Tetracosane:CH ₃ -(CH ₂) ₂₂ -CH ₃	91 ⁴ ,97 ⁵	98 ¹⁰ ,98 ¹⁰ ,93 ¹⁰ , ,96 ¹² ,96 ¹⁴		
14	2-Methyl octacosane CH ₃ -CH(CH ₃)-(CH ₂) ₂₅ -CH ₃	90 ⁵			
15	Octadecane:CH ₃ -(CH ₂) ₁₆ -CH ₃	93 ⁷ ,92 ⁸	96,93 ¹³ ,91 ⁵	94 ¹⁰ ,92 ³³ , 93 ²⁸ ,91 ³⁸ , 93 ³⁹ ,87 ³⁹	11

16	Tetratetracontane: $\text{CH}_3-(\text{CH}_2)_{42}-\text{CH}_3$.	93 ⁷	92,87 ¹³		91 ¹¹ ,91 ²⁶	4
17	Heneicosane: $\text{CH}_3(\text{CH}_2)_{24}\text{CH}_3$					
18	Nonadecane: $\text{CH}_3-(\text{CH}_2)_{17}-\text{CH}_3$		90 ⁹		90 ³⁸ ,91 ³¹ , 91 ³⁴ ,91 ¹⁰ ,96 ¹³ ,95 ³⁶ ,	7
19	Styrene			96 ⁵	93 ¹³	
20	 Styrene			91 ⁵	96 ⁵	
21	 Bicyclo[4.2.0]octa-1,3,5-triene				94 ⁵	
22	Styrenene					
23	 alpha,-pinene			96 ⁶ ,91 ⁶	90 ⁴	
24	 (1R)-2,6,6-Trimethyl bicyclo[3.1.1]hept-2			94 ⁶	94 ⁴	

25	Hept-2-ene			91 ⁶		
26	Eucalyptol			91 ⁹		
27	1,3,5,7-Cyclooctatetrane			94 ⁴	94 ³	
28	Dodecane			70 ¹⁴ ,72 ¹⁵		
29	Bicyclo[3.1.1]heptane-6,6-dimethyl[-2-methylene-(15)-				87 ⁴	
35	2-methyloctacosane				91 ²⁶	
36	Octacosane				81 ⁸	
37	Sulfurous acid, butyltetradecene ester				91 ¹¹	
38	Dodecane, 2-methyl				89 ¹¹	
39	Decane, 3,8-dimethyl				91 ¹³	
40	Hexadecane, 1-iodo				92 ³¹	
41	Heptacosane, 1-chloro		87 ¹³		91 ³¹	
42	Tritetracontane				91 ²⁸ , 91 ³¹	
43	Pentadecane				93 ³² , 90 ³⁶ 93 ³²	
44	3-Eicosene(E) 				92 ³³	

45	Eicosane	95 ⁶ ,95 ⁷ ,94 ⁶ , 95 ² ,90 ³	95 ¹² ,95 ¹⁵ , 91 ¹⁵		95 ¹⁶ ,91 ¹⁶ , 93 ²⁹ ,72 ²⁹ , 93 ³² ,98 ³⁴ , 86 ³⁹ ,91 ³⁴ , 98 ³⁶	17
46	Hexadacane,1-iodo				92 ³¹	
47	Heptacosane,1-chloro				91 ³¹	



Type of extract	Mg extract concentration	P.m Zone of inhibition	S.a Zone of inhibition	E.c Zone of inhibition	Pseu. a Zone of inhibition	Remark
A-4	1.5	6	6	6	6	
A3	0.5	6	6	6	6	
A2	1.5	6	6	6	6	
R2	0.5	6	6	6	6	
R3	1.5	6	6	6	6	
B-7	1.5	6	9	12	6	
A5	0.5	6	6	6	6	
ATO	1.5	6	7	13	6	
Da	0.5	6	6	6	6	
Df	1.5	6	15	6	6	
	0.5	6	6	6	6	



Control for

Pseudomonas 6m

S .aureus 6m

Before actions

That is the chloroform doesn't affect the growth of organism in that it may evaporate immediate

Sign: ~~Tabene Abdissa~~
 Chief Medical Laboratory
 Tech. II & B.Pharm

APPENDIX 15



OROMIA PUBLIC HEALTH RESEARCH , CAPACITY BUILDING & QUALITY ASSURANCE LABORATORY

ADAMA ,ETHIOPIA

ANTIBIOTIC QUALITY CONTROL - WEEKLY GRAM-POSITIVE

Date performed: 13/12/2008 Tech Initial: EF

SN	ANTIBIOTIC	CODE	CONC (mcg)	Manuf acturer Name	Lot#	Open Date	Expire Date	ACCEPTAB LE LIMITS (mm)	Results		
									Zone size (mm)	Acceptab le? Yes (✓) No (X)	Remar
1	CEFOXITIN	FOX	30					23 - 29	26mm	✓	
2	CLINDAMYCIN	DA	10					24 - 30	27mm	✓	
3	ERYTHROMYCIN	E	15					22 - 30	25mm	✓	
4	OXACILLIN	OX	1					18 - 24	23mm	✓	
5	PENICILLIN	P	10 U					26 - 37	30mm	✓	
6	TETRACYCLINE	T	30					24 - 30	27mm	✓	
7	TRIMETH - SULFA	SXT	25					23 - 29			
8	VANCOMYCIN	VA	30					17 - 21	17mm	✓	

Mueller Hinton Agar (MHA) Preparation date: Expiry date:

Tech Initial: EF Date: 14/12/2008 Supervisor Initial: Date:

Test Organism: *S. aureus* 25923 on Mueller Hinton medium. Incubate at 35°C ambient air for 16 - 18 hours.

QC Frequency: Perform QC on each new lot number or shipment and weekly thereafter. Refer to AST SOP for test procedure.

If QC failed, do not use lot and do not report patient results. Inform supervisor immediately. Document all QC failure and corrective action taken.

QC Failure/ Corrective Action All Qc disk size is with in the acceptable range



OROMIA PUBLIC HEALTH RESEARCH, CAPACITY BUILDING & QUALITY ASSURANCE LABORATORY

ADAMA, ETHIOPIA

ANTIBIOTIC QUALITY CONTROL - WEEKLY GRAM-NEGATIVE

Date performed: 13/12/2008

Tech Initials: EF

SN	ANTIBIOTIC	CODE	CON C. µg/ml	Manuf acturer Name	LOT #	Open Date	Expiry Date	ACCEPTA BLE RANGE (mm)	RESULTS	
									Zon e size (mm)	Accepta ble? Yes (✓) No (X)
1	AMIKACIN	AK	10					19-26	19mm	✓
2	AMPICILLIN	AMP	10					16-22	22mm	✓
3	AMOXYCILLIN	AML	25							
4	AMOX-CLAV ACID	AMC	30					18-24	19mm	✓
5	CEFAZOLIN	KZ	30					21-27	21mm	✓
6	CEFEPIME	FEP	30					31-37		
7	CEFOTAXIME	CTX	30					29-35	30mm	✓
8	CEFTAZIDIME	CAZ	30					25-32	29mm	✓
9	CEFTRIAZONE	CRO	30					29-35	30mm	✓
10	CEFUROXAME	CXM	30					20-26	26mm	✓
11	CEPHALOTHIN	KF	30					15-21		
12	CHLORAMPHENICOL	C	30					21-27	25mm	✓
13	CIPROFOXACIN	CIP	5					30-40	31mm	✓
14	GENTAMICIN	GM/CN	10					19-26	20mm	✓
15	IMIPENEM	IMP	10					26-32	28mm	✓
16	NALIDIXIC ACID	NA	30					22-28	24mm	✓
17	NITROFURANTOIN	NI	300					20-25		
18	PIPERACILLIN	PRL	100					24-30		
19	PIPERACILLIN/TAZOB ACTAM	TZP	110					24-30		
20	TOBRAMYCIN	TOB	10					18-26	20mm	✓

MHA Preparation date:
Supervisor Initials

Expiration Date:
Date:

Reading Tech Initials

Date

EF

14/12/2008

QC Organism: E. coli ATCC 25922 on Mueller Hinton medium. Incubate at 35°C ambient air for 16 – 18 hours.

QC Frequency: Perform QC on each new lot number or shipment and weekly thereafter. Refer to AST SOP for test procedure.

if QC fails, do not use lot and do not report patient results. Inform supervisor immediately. Document all QC failures and corrective action taken.

QC Failure/ Corrective Action: All Qc disk size is within the acceptable range.