Addis Ababa University

College of Natural and Computational Sciences

School of Graduate Studies, Department of Chemistry

PhD Dissertation

Antifungal Natural Products against two Plant Diseases: Root rot/wilt of Faba Bean and Late Blight of Potato

By: Minbale Gashu

Advisor: Professor Ermias Dagne (Addis Ababa University)

Co-advisor: Dr. Melaku Alemu (Ethiopian Institute of Agricultural Research-Ambo Plant Protection Research Center)

August, 2018

Addis Ababa, Ethiopia

Approval Sheet

Addis Ababa University
College of Natural and Computational Sciences
School of Graduate Studies
Department of Chemistry

This is to certify that the dissertation prepared by Minbale Gashu Tadesse, entitled: Antifungal Natural Products against two Plant Diseases: Root rot/wilt of faba bean and Late blight of potato, submitted in partial fulfillment of the requirement of the degree of Doctor of Philosophy in Chemistry (organic stream) complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

Approved by the Examining Committee:

External examiner

Erik Alexandersson (Professor)

Internal Examiner

Wendimagegn Mammo (Professor)

Internal Examiner

Estifanos Ele (PhD)

Advisor

Ermias Dagne (Professor)

Co-advisor

Melaku Alemu (PhD)

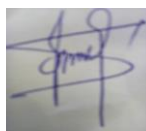
Chairman of the Department

Ahmed Mustefa (PhD)

Declaration

I hereby declare that this Disertation is submitted for the degree of Doctor of Philosophy (PhD) in Chemistry (Organic stream) at Addis Ababa University, College of Natural and Computational Sciences, Addis Ababa, Ethiopia, is my own original work and has not been submitted previously to any institution or university. Resources and materials used in this work have been duly acknowledged.

Name: Minbale Gashu

Signature: 

Date: August, 2018

Table of Contents

Contents **Page**

Approval Sheet ii

Declaration iii

Acknowledgements vi

Abstract vii

Acronyms and abbreviations viii

1. Introduction 1

1.1 Production of Faba bean and Potato in Ethiopia 2

1.2 Severity and characteristics of root rot/wilt and late blight in Ethiopia 2

1.3 Methods of combating root rot/wilt and late blight 4

1.4 Use of natural products to control root rot/wilt and late blight 7

1.5 Main strategy of this project 8

1.5.1 Review of literature on ethnobotany, biological activities and chemistry of *Inula spp.* 8

1.5.2 Traditional uses and chemistry of the *Echinops spp.* 30

1.5.3 Review on Chemistry of *Artemisia annua* Leaf 34

1.5.4 Essential oil composition of *Eucalyptus globulus* and *Thymus schimperi* Leaf 35

1.6. Objectives of this study 36

2. Results and Discussion 38

2.1 Optimization of the *in vitro* antifungal assay: Food poisoning method 38

2.2 Screening of plants against *Fusarium spp.* and *Phytophtra infestans* 39

2.2.1. Mycelial growth inhibition potential of plant extracts and essential oils 39

2.2.2. Fractionation of selected plant extracts for bioassay guided chemical study 44

2.3 Characterization of compounds and essential oils 45

2.3.1 Characterization of compounds isolated from *Inula confertiflora* 45

2.3.2 Identification of chemical constituents of essential oils from *I. confertiflora* 63

2.3.3 Characterization of dehydrocostus lactone isolated from *E. Kebericho* root and its modified analogues 65

2.3.4. Characterization of compounds isolated from *Artemisia annua* leaf 73

2.3.5 GC-MS analysis of leaf essential oils from *Artemisia annua*, *Eucalyptus globulus* and *Thymus schimperi* 78

2.4 *In vitro* antifungal activity of isolated and modified compounds 79
2.4.1 Antifungal activity of compounds isolated from *Echinops kebericho*, *Inula confertiflora* and *Artemisia annua* 80
2.4.2 Comparison of antifungal activity of DHCL and its derivatives 80
3. Experimental Part 82
3.1 Chemicals and equipment 82
3.2 List of plants collected for this study 82
3.3 List and isolation of plant fungal pathogens used in this study 84
3.4 *In vitro* antifungal activity assay (Food Poisoning)and statistical analysis 85
3.5 Extraction and fractionation of plant extracts for antifungal and chemical study 86
3.6 Isolation, purification and structure elucidations of compounds 86
3.7 Isolation, analysis and identification of components of Essential oils 97
4. Conclusion and Recommendations 98
5. References 99
Appendix: NMR spectra of compounds 113

Acknowledgements

I express my gratitude to my principal advisor Professor Ermias Dagne (Chemistry Department, AAU) for his supervision and guidance during the course of this study, and for his help in collecting plant specimens from Ankober Palace Lodge. I am also thankful to my co-advisor Dr. Melaku Alemu (Ambo Plant Protection Research Center, APPRC) for his guidance and encouragement.

I also thank Professor Wendimagegn Mammo for generating NMR data & guidance in structure elucidations, and for his support to synthesize compounds. It is also my pleasure to thank Dr. Estifanos Ele and Mrs. Senait Dagne for GC-MS analysis, and Dr. Yonas Chebude and W/o Woinshet Gebeyehu for IR analysis. I also appreciate support of Dr. Aman Dekebo and Dr. Jung in Korean Research Institute of Chemical Technology for LC-MS analysis, and Prof. Yang Ye of Shanghai Institute of Materia Medica (Chinese Academy of Sciences) for MS analysis.

Former directors of Ambo Plant Protection Research Center (APPRC) of Ethiopian Institute of Agricultural Research (EIAR) especially Ato Tariku Hunduma and Ato Endale Hailu, and all mycology staff members, namely, Ato Gudisa Hailu and Ato Medeksa Fufa are appreciated for their support while conducting bioassay work.

Dr. Ahmed Mustefa (Chairman of the Chemistry Department, Addis Ababa University) is thanked for his cooperation in administrative works. Debre Berhan University is thanked for study leave. Dr. Ermias Lulekal and Ato Melaku Wondafrash of the National Herbarium of Ethiopia (AAU) are acknowledged for plant identification.

It is my great pleasure also to express my deep gratitude to my immediate family: my wife Almaz Awoke and my son Fiqir Minbale.

Abstract

Antifungal Natural Products against two Plant Diseases:
Root rot/wilt of Faba Bean and Late Blight of Potato

By: Minbale Gashu

Advisor: Professor Ermias Dagne (Addis Ababa University)
Co-advisor: Dr. Melaku Alemu (Ambo Plant Protection Research Center)

In Ethiopia faba bean and potato are two important crops for food security and nutrition. Root rot/wilt is a notorious fungal disease of faba bean caused by *Fusarium spp*., while late blight is a potato disease due to attack by the fungus *Phytophtra infestans.* These two diseases cause from time to time severe crop losses. Hence it is important to control growth and spread of these fungal diseases. It is well known that although the use of synthetic fungicides contributes to enhance crop yield, toxic residues in treated crops cause environmental and health problems. Therefore, the search for eco-friendly plant-based antifungal agents is a worthwhile strategy.

In this work, the two pathogenic fungi were isolated from diseased parts of crops, identified and maintained with periodic sub-culturing in Potato Dextrose Agar. In this study fifty seven ethanol extracts of different plants were screened for their antifungal activity using optimized food poisoning technique. Dimethylsulfoxide (5% aq) and 0.5% Mancolaxyl were used as vehicle and standard, respectively. The standard fungicide showed respective growth inhibition percentage (%GI) of 68 and 88 against *F. spp.* and *P. infestans* at a concentration of 5 mg/mL. Bioactivity of screened plant extracts was classified into high, moderate and inactive by taking the minimum %GI of the standard (68%) as reference. Of these extracts, *Inula confertiflora* (69/78%), *Echinops kebericho* (73/80%) and *Artemisia annua* (71/73%) showed higher %GI in the control of *F. spp.* / *P. infestans*, respectively. The nonpolar portions were found comparable with that of the reference in inhibiting mycelial growth of tested fungi relative to their polar counterparts. The active compounds were isolated and characterized. In the bioassay study dehydrocostus lactone, carabrone, graveolide, carpesiolin and artemisinin demonstrated significant inhibition on the growth of tested fungi.

Acronyms and abbreviations

AAU	Adds Ababa University
APPRC	Ambo Plant Protection Research Center
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
CC	Column Chromatography
COSY	Correlated Spectroscopy
CSA	Central Statistics Authority
d,dd,s,t,m,br	doublet, double doublet,singlet, triplet, multiplet, broad resonances
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
DW	Distilled Water
EIAR	Ethiopian Institute of Agricultural Research
GC-MS	Gas Chromatography coupled with Mass Spectrophotometer
GDP	Gross Domestic Product
^{1}H- NMR	Proton Nuclear Magnetic Resonance
Ha	Hectare
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IDM	Integrated Disease Management
IR	Infra-Red
LC/EI-MS	Liquid Chromatography/Electron Ionization Mass Spectrophotometer
Mp	Melting point
NDA	Natural Data Base for Africa
NPC	Natural Product Chemistry Project
t/q	tone/quintal
TLC	Thin Layer Chromatography
UV-Vis	Ultra Violet- Visible
DNP	Dictionary of Natural Product
EO	Essential oil

1. Introduction

Agriculture is the mainstay of the Ethiopian economy. This sector accounts for more than 40% of the GDP, 80% of exports and over 80% of the country's workforce [1]. The leading export crops include coffee (17%), vegetables (10%), oilseeds (9%) and pulses (4%) [2].

Pulses are the edible seeds of plants of the legume family (Fabaceae). In Ethiopia pulses are important plant-based source of protein accounting for 15% of all protein intakes, and also playing significant role in export and soil fertility [3]. Main pulses of Ethiopia include faba bean (*Vicia faba*), chickpea (*Cicer ariethinum*), common bean (*Phaseolus vulgaris*), field pea (*Pisum sativum*) and lentil (*Lens culinaris*). The Amharic names of these common pulses are *Baqela* (faba bean), *Shimbera* (chickpea), *Boloqe* (common bean), *Ater* (field pea) and *Misir* (lentil). Over 8 million Ethiopian peasant households are engaged in pulse production covering about 2 million ha of land with produce of approximately 27 million quintals per annum [3]. Faba bean is the leading pulse crop cultivated in Ethiopia serving as the main protein source for majority of the population.

Vegetables with edible roots and tubers are categorized as root and tuber crops. The most important root and tuber crops in Ethiopia include *Enset* (*Ensete ventricosum*), potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*). Potato is the fourth most important food crop in the world after wheat, maize and rice. It is a popular and affordable non-grain source of carbohydrates, high quality proteins and essential vitamins and minerals in the world [4, 5]. It produces more food per unit area than other major food grains and serves as an important source of income for farm households [6-8]. Potato was introduced to Ethiopia by the German botanist, Wilhelm Schimperi in 1858 (Richard Pankhurst, 1964).

Increasing crop yield by managing major plant threats (pests) is important for improving food availability and nutrition security in developing countries. The purpose of this work is to find antifungal compounds against devastating fungal diseases of faba bean and

potato in Ethiopia. The production of the crops under study, their major diseases and control measures are discussed below.

1.1 Production of Faba bean and Potato in Ethiopia

Ethiopia is the second largest producer of faba bean in the developing world next to China. It contributes 7% of the world production and 40% of that of Africa [9]. Faba bean occupies the largest share of the land (4%) and annual production (3%) of pulses keeps on growing in Ethiopia. On the other hand the average yield of pulses in Ethiopia is about 1 t/ha, which is lower than the world average of 1.8 t/ha [3].

Potato is the most important food crop, ranking first in volume of production among root and tuber crops in Ethiopia. It is easily accessible and widely consumed food crop. However, the local average yield of potato (10 t/ha) is below world average (15 t/ha) [6].

Yield of faba bean and potato is affected by changes in temperature, moisture, mineral nutrients and drought. Substantial amount (36%) of all food crops produced in the world is damaged annually by diseases, insects and weeds; of which about 14% of food is lost due to plant diseases caused by fungi, viruses, bacteria and nematodes. As compared to other plant parasites fungi cause the greatest influence on crop production [10]. Fungal diseases that damage faba bean and potato are presented below (Table 1).

1.2 Severity and characteristics of root rot/wilt and late blight in Ethiopia

Diseases that cause major economic losses in the production of faba bean in Ethiopia are fungal in origin. These are black root rot/wilt (*Fusarium spp.*), rust (*Uromyces viciae-fabae*), faba bean gall (*Olpidium viciae*) and chocolate spot (*Botrytis fabae*) (Table 1). Root rot/wilt affects largely pulses of which the effect is significant (up to 60% loss) in faba bean production in Ethiopia [3, 11-14].

Root rot/wilt is a complex disease that causes crop death after severe root-rotting and wilting. The disease occurs exclusively in black soils rich in clay (vertisols) where water logging is critical. It is a key factor that predisposes faba bean to the disease. Clay-rich soils contain little organic matter and therefore shrink as they dry and swell when they

become wet. Gradually, infected crops lose their normal green coloration of the leaves, show dark black roots and finally disintegrate. Root rot/wilt-causing fungi can survive for long periods of time in the soil in the form of resting spores called chlamydospores. These spores have high potential to cause disease on newly emerging faba bean crops [3]. This makes the disease difficult to manage.

Table 1. Major diseases of faba bean and potato in Ethiopia [3, 6]

Pathogen	**Disease name**	**Type**	**Host crop**
Alternaria tenuis	Black rot *		
Ascochyta fabae	Blight*		
Rhizoctonia solani	Wet root rot*		
Fusarium avenaceum	Foot rot**		
Uromyces viciae-fabae	Rust***	Fungus	Faba bean
Botrytis fabae	Chocolate spot ***		
Fusarium solani	Black root rot ***		
Fusarium spp. and R.solani	Root rot/wilt***		
Meloidogyne incognita	Root knot*	Nematode	
Phytophthora infestans	Late blight***		
Alternaria solani	Early blight***		
Ascochyta hotorum	Leaf spot*		
Rhizoctonia solani	Root rot*		
Sclerotium rolfsii	Root rot*	Fungus	Potato
Macrophomina phaseoli	Charcoal rot*		
Fusarium coeruleum	Dry rot*		
Ralstonia solanacearum	Bacterial wilt***	Bacteria	
Meloidogyne javanica	Root knot***	Nematode	

Severity of the diseases: *=Minor **=Medium ***=Major

Production of potato in Ethiopia is very much affected by the fungal disease known as late blight (*Phytophthora infestans)* and bacterial wilt (*Ralstonia solanacearum*) (Table 1). Although the fungus is likely to infect plants in the Solanaceae family, it is commonly encountered in tomato and potato plantations in the world [6]. The fungus causes estimated world potato tuber yield loss of about 25-35% [6, 7]. In Ethiopia, the disease occurs throughout the major potato producing areas and causes up to 90% tuber yield loss in cool and wet season due to both premature death of foliage and diseased tubers [8, 15].

The fungus, *P. infestans,* stays alive under adverse conditions and throughout winter in the form of resting spores called oospores. The oospore is dispersed through soil, water, wind and then starts to invade, grow and infect new potato seedlings in the field [7, 8].

Root rot/wilt and late blight are among widely distributed and destructive diseases of faba bean and potato in Ethiopia, respectively. The significant loss of the crops by these diseases can impair their production in the main rainy season (*Kiremit*) without fungicide applications [3, 7, 15].

1.3 Methods of combating root rot/wilt and late blight

In general there are six major methods of controlling plant pests; that is, cultural/ physical, biological, host plant resistance, chemical, botanical and integrated pest/ disease management system [10]. The methods that are used in managing root rot/wilt of faba bean and late blight of potato are highlighted below.

Cultural and Physical methods

Cultural methods of controlling plant diseases focus on avoiding contact between plants and pathogen, creating environmental conditions unfavorable to the pathogen and eradicating or reducing the amount of pathogen around the host plant [3, 6].

Cultural practices that are used to treat root rot/wilt of faba bean comprise sanitation, proper drainage of fields and rotation with crops that are not-hosts of *Fusarium spp*. such as *Guizotia abyssinica (Nug), Brassica napus (Gomen zer*) and *Linum usitatissimum* (*Telba*) [3, 16]. These can reduce inoculum level of the pathogen in the soil.

Discarding waste potatoes before crop emergence, use of disease-free seed, keeping foliage dry, avoiding dense planting, removing and destroying affected plants and weeds are applicable cultural methods to minimize late blight risks [7, 8].

The development of diseases is strongly associated with soil temperature besides moisture, inoculum level of the specific pathogen in the soil and crop age. The physical method used commonly in controlling pre-harvest plant fungal diseases including the targeted diseases is soil solarisation [10]. It is a nonchemical method for controlling soil borne pests using high temperatures produced by capturing radiant energy from the sun.

Biological control methods

Biological control is the total or partial destruction of target pathogen using living organisms, particularly its natural enemies or parasites. Use of such natural antagonistic microorganisms is considered as economically and ecologically selective management method of plant diseases. The mechanism of action is either competition for substrate in the rhizosphere or produces inhibitory chemicals and induces systemic resistance in host plants [17, 18].

Fungi, viz., *Trichoderma pseudokoningii, T. harizianum, Bacillus megaterium var*. *phosphaticum*, *B. subtilis* and *Pseudomonas fluorescens* are commonly used biocontrol agents for treating plant pathogenic fungi. In Ethiopia, *T. viride* and *P. fluorescens* are studied for treating root-rot and late blight, respectively [3, 6, 13, 17-19].

Host plant resistance

It is a type of disease controlling method involving the transfer of genetic material into host plants and the production of genetically modified plants that exhibit resistance to a certain disease. Resistant varities can also obtained by inducing the crop to produce defence metabolites in response to infection. The use of such resistant cultivars is widely recognized as the safest and most effective sustainable method for protecting crops from diseases such as root rot/wilt and late blight [20].

The studies conducted by the National faba bean improvement program at Holeta agricultural research center, Sheno aricultural research center and APPRC identified black root rot disease resistant varieties (Wayu (Or), Selale (Or), Lalo (Amh), Dagm (Amh)) producing high yield and released to farmers [3]. Cultivation of resistant potato varieties (Eg Tolcha (Or)) was reported to produce higher tuber yield than the local variety due to its potential to resist late blight [6].

Chemical Methods

Chemical methods involve the direct use and action of synthetic fungicides to reduce or kill fungi population. They are applicable as foliar sprays and dusts, seed treatments, soil treatments and as soil fumigants [3].

In developing countries the use of chemical fungicides/ pesticides account for 20-26% of the world's pesticides market [10]. Fungicides commercialized as Benomyl™, Rizolex-T™, Redomil MZ™ and Mancozeb™ are commonly used chemicals to control soil borne fungi in different crop farms including faba bean [3]. The active ingredients they contain are benzimidazole (**1**), tolclofos-methyl chlorophosphate (**2**), metalaxyl phenylamide (**3**) and ethylene bis (dithiocarbamate (**4**), respectively (Fig. 1).

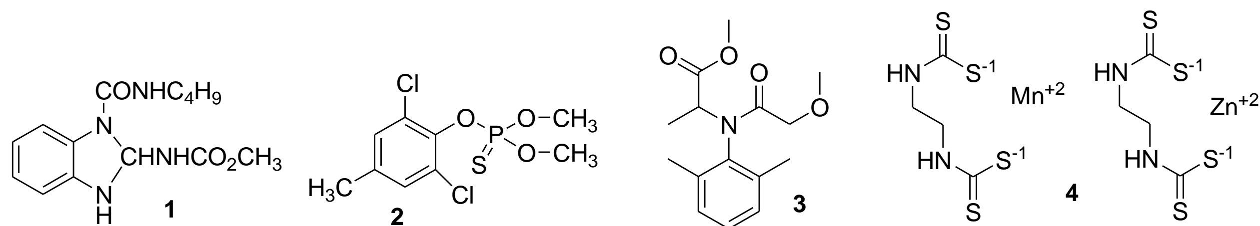


Fig.1 Active ingredients of some fungicides

Chemicals sold in the name of Unizeb™, Ridom™, Mancozeb™ (**4**) and Redomil MZ™ (**3**) are also applied to control late blight of potato. In addition, Chlorothalonil (2, 4, 5, 6-tetrachlorobenzene-1, 3-dicarbonitrile) and Brestan 10 (triphenyltin acetate) are used to treat fields with late blight infestations [3, 6-8].

Repeated use and misuse of fungicides induce resistance in plant pathogens, cause acute toxicity, accumulate in the food chain due to their long degradation periods and have a power to pollute both useful organisms (Eg. bees) and harmful organisms. These effects seriously hinder the management of crop diseases involving synthetic chemicals [3, 6, 10, 15].

Integrated disease management method (IDM)

Generally, IDM is a strategy that combines all available methods of controlling pests to ensure the healthy growth of crops. It is the use of combination of two or three of the techniques such as use of resistant cultivars, sowing certified seeds, choosing fields with low inoculum levels, utilization of antagonistic microbes, applying plant based products and sometimes use of chemicals as required [21-25].

1.4 Use of natural products to control root rot/wilt and late blight

Natural product-based fungicides are generally recognized as safe in agriculture and emerge as one of the alternative methods to protect crops and their products and the environment from fungicide pollution [21, 26, 27].

Recent reports [21] indicate several plant extracts possess antifungal properties in particular against growth of *Fusarium spp.* and *P. infestans* (Table 2). Upon their chemical studies, azadirachtin (**5**) [27, 28], vanillin(**6**) [29], pinoresinol (**7**) [29], betulin (**8**) [30, 31], β-caryophyllene (**9**) [30-33], parthenin (**10**) [34-36], 5'-(3-buten-1-ynyl)-2, 2'-bithiophene (**11**) [34, 35] and withaferin (**12**) [27] (Fig. 2) were isolated as active constituents.

Table 2. Plants with antifungal properties against *Fusarium spp.*[i] and *P. infestans* [ii]

Scientific name (Amharic name)	**Part used [pathogen]**	**Isolated compounds**	**Ref**
*Azadirachta indica (*Neem)	FR/SD/LF [i]	azadirachtin	27, 28, 36-39
Melia azaderach	SD/LF [ii]	vanillin, pinoresinol	29, 40
Croton macrostachys (*Bisana*)	BK [ii]	betulin,12-oxo-hardwickic acid	30, 31, 41
Hagenia abyssinica (*Kosso*)	FL, WD [i,ii]	kosins (phloroglucinol derivatives)	27, 31
Maesa lanceolata (Abayi)	BK, LF, FR[i]	triterpenoid saponins	42
Lantana camara (*Yewef qolo*)	LF [i,ii]	sesquiterpene hydrocarbons, mainly β-caryophyllene	30-33, 43
Parthenium hysterophorus (*Qenche arem*)	LF [i,ii]	Parthenin	33, 44, 45
Ricinus communis (*Gulo)*	LF [i]	flavonoids, coumarin, ricinin	27
Tagetes minuta (*Yahiya shito*)	LF [i]	acetylinic thiophenes	34, 35
Oxalis corniculata (Yefyel chew)	LF and FR	-----	46
Balanites aegyptiaca (*Bedeno*)	LF, FR [i]	saponin (balanitesin)	47
Ocimum gratissimum (Checho)	LF [i]	Terpenoids	46, 48
Withania somnifera (Gezawa)	LF [i,ii]	lactone	27
Lepidium sativum (Feto)	SD [ii]	----	31, 49
Cupressus benthamii	LF [ii]	----	30
Vetiveria zizanioides	RT [ii]	phenolic acids	30, 50, 51
Jatropha curcas	SD [ii]	-----	52, 53

FR= fruit, SD=seed, BK= bark, LF= leaf, RT= root, FL= flower, WD= wood

The essential oil and methanol extract of *Metasequoia glyptostroboides* (Cupressaceae) also showed high (49–70%) inhibition of mycelial growth of *F. oxysporum*, *F. solani* and *Phytophthora sp* [21].

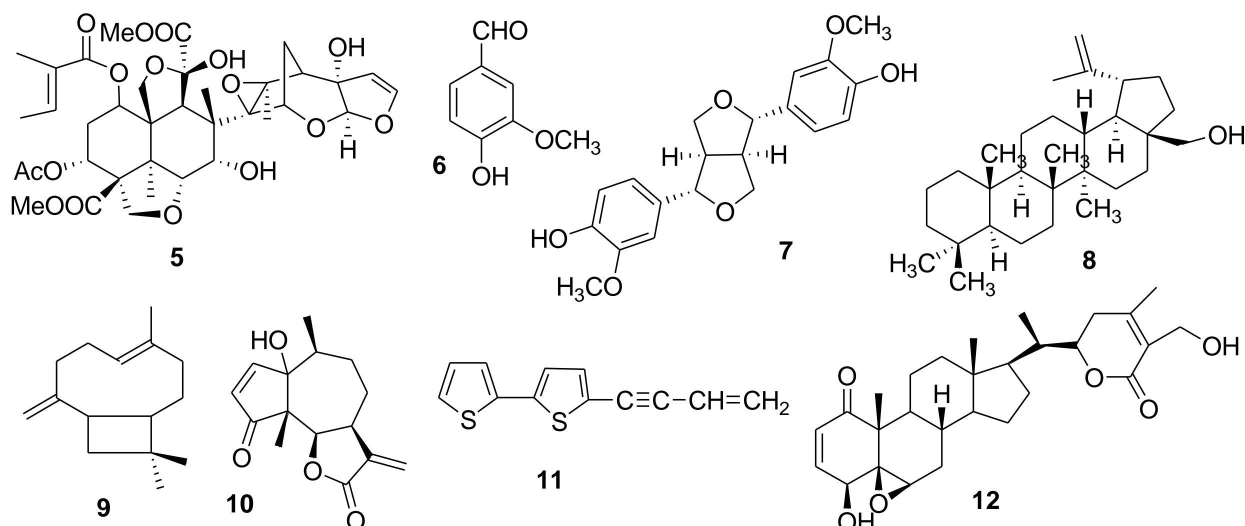


Fig. 2 Chemical structures of some active compounds isolated from listed plants

Despite the positive indications, to the best of our knowledge plant extracts or isolated compounds are still not in use to control fungal diseases caused by *F. sp* and *P. infestans*.

1.5 Main strategy of this project

Based on literature reports and availability of species several plants were collected and extracted. Plant species under study were selected for intensive studies based on their preliminary antifungal activity test against *F. sp* and *P. infestans* done as part of this work. In the course of screening plant extracts weak to strong antifungal activities were observed and of these screened plants *Echinops kebericho, Inula confertiflora, Artemisia annua, Eucalyptus globulus* and *Thymus schimperi* were selected for investigation. We, therefore, undertook thorough chemical literature search on traditional uses, biological properties and chemical constituents of plants investigated and reviewed. These reviews are presented as follows.

1.5.1 Review of literature on ethnobotany, biological activities and chemistry of *Inula spp*.

The Inula genus (tribe Inuleae) belongs to Asteraceae (Compositae) family. It is composed of more than 100 species distributed in Asia, Europe and Africa. They are

mostly herbs with alternate leaves, sometimes with an offensive smell [54-56]. There are many studies reported on ethnopharmacological aplications of various *Inula spp*. [54-56].

In China Traditional Medicine *I. helenium* (elecampane), *I. racemosa* and *I. britannica* are used for expectorants, antitussives, diaphoretics, antiemetics, and bactericides [54, 55]. *I. helenium* and *I. japonica* are used in Chinese herbal preparations namely Huang-Lian-Shang-Qing tablets and antiulcerous drug ventrofit, respectively [56].

In Chinese folk medicine, *I. hupehensis* is used to treat bronchitis, diabetes and intestinal ulcers and *I. cappa* is applied for treating rheumatoid arthritis, dysentery, hepatitis and jaundice. Another *Inula* species named *I. helianthus-aquatica* is used to care for cancer [54, 56].

I. racemosa is used in Pakistan traditional medicine against hypertension and, *I. obtusifolia* and *I. racemosa* are also reported to show antihelmintic, antiseptic, expectorant, diuretic, and hypotensive activities. In Indian traditional medicine, *I. racemosa* is used as an expectorant, bronchodilator, to treat tuberculosis and skin diseases. The root powder of the plant is also reported for handling asthma-like conditions, to reduce cholesterol, to support healthy circulation, to treat angina and dyspnea and to ensure healthy heart functions. It is also used for the management of diabetes [54-56].

The Chilandar Medical Codex *I. helenium* is used to treat wounds and dandruff, bronchitis and bronchial asthma. In Hungarian traditional medicine the essential oil is used to treat respiratory and digestive diseases and the roots are used against asthma, cough, bronchitis, lung disorders, tuberculosis, indigestion, infectious and helminthic diseases [54-56]. People in Morocco,Jordan and Palestine used *I. viscosa* (Syn. *Dittrichia viscosa*) for its anti-inflammatory, antipyretic, antiseptic, antiphlogistic, antihelmintic, diuretic, anti-anemic, anti-scabies activities and to treat gastro-duodenal disorders and infertility. The roots of *I. viscosa* are also used against cough, to treat hypertension and diabetes mellitus. Decoction of *Inula viscosa* roots were also used in the treatment of skin irritations of allergic origin in Italian traditional medicine [54-56]. In China, reports on traditional uses of *I. britannica* indicated its use for the treatment of

asthma, chronic bronchitis and acute pleurisy and *I. japonica* is applied to treat furunculosis, cough, digestive disorders, bronchitis and inflammation. The flowers of *I. britannica/ I. japonica* are also used to treat digestive disorders, bronchitis and inflammation, bacterial and viral infections [54, 55, 57].

Ethnopharmacological study conducted elsewhere indicated that *I. confertiflora* is found applicable to treat skin diseases caused by virus, wounds and eczematous lesions [58]. In Ethiopian rural areas, dried roots of *I. confertiflora* are smoked as a fumigant during child birth. The maceration of pounded leaves in water is also applied to diseased eyes of cattle. Traditional healers in Ethiopia used the roots and flower of the plant against leprosy, and the leaf for treating asthma, common cold and cough [59].

Chemistry of *Inula* species

Phytochemical constituents of some species, namely, *I. britannica, I. helenium, I. japonica, I. viscosa, I. racemosa, I. macrophylla* and *I. cappa* were well investigated. The chemical compounds isolated from Inula species include monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, flavonoids and glycosides. These Inula constituents have been demonstrated to exert diverse biological activities, particularly, cytotoxic and antitumoral and antiinflamatory. Of all the isolated compounds sesquiterpene lactones were found to be the major constituents [54-56].

Sesquiterpene lactones are a large group of naturally occurring secondary metabolites that have been isolated from several plants, mostly from the Asteraceae family. They show diversity of chemical structures where eudesmanolides (**13**), guaianolides (guai-11(13)-en-12,8-olide (**14**), guai-11(13)-en-12,6-olide (**16**)), pseudoguianolides (pseudoguai-11(13)-en-12,8-olide (**15**), pseudoguai-11(13)-en-12,6-olide (**17**)), germacranolides (**18**) and xanthanolides (**19**) (Fig. 3) are the most representative classes. Sesquiterpene lactones of these groups displayed a wide range of biological properties including anticancer, anti-inflammatory, antifungal and antibacterial activities [54-56, 60].

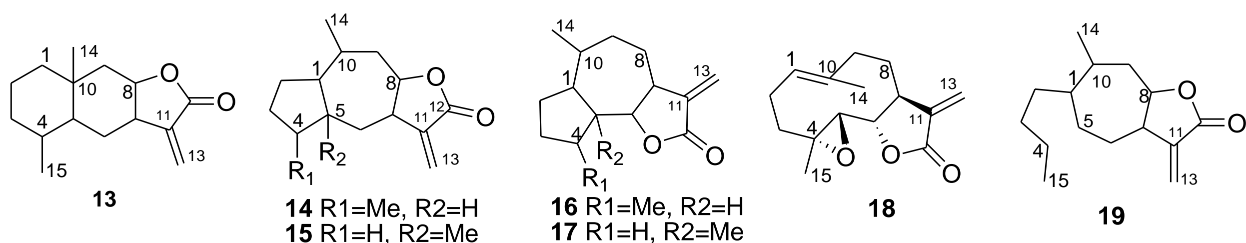


Fig. 3 Representative structures of classes of sesquiterpenes lactones

Sesquiterpene lactones of eudesmanolide-type were reported as the most common compounds in *I. helenium, I. racemosa, I. hupehensis, I. britannica* and *I. britannica var. chinensis*. Alantolactone (**20**) was the first sesquiterpene lactone to be isolated from Inula species. It is a well known eudesmanolide-type sesquiterpene lactone for the allergic contact dermatitis induced by the plant species. The constitutional isomer isoalantolactone (**21**) and many others (**22**-**106**) were also isolated from many Inula species [54, 56].

The other usually encountered classes of sesquiterpene lactones in Inula species are guaianolides (**107**-**128**) and pseudoguaianolides (**129**-**166**). They are responsible compounds for the anticipated biological activities demonstrated by the source plants. For instance the anti-inflammatory activity of *I. viscosa* is attributed to the presence of inuviscolide (**107**). The anticancer property claimed for *I. helianthus-aquatica* leaves and flowers was demonstrated by promising inhibitory activities of bigelovin (**132**) against the growth of several cancer cell lines [54, 56].

Xanthanolides (**167-191**) are groups of sesquiterpene lactones which are found in Inula species. They are biogenetically formed from the guaiane skeleton by ring opening at C(4)/C(5). Tomentosin (**167**) is the most common xanthanolide type sesquiterpene lactone isolated from several species [54, 56] and the antifungal activity of *I. viscosa* against downy mildew of grapes was credited to it and costic acid (**279**). These bioassay results showed the potential of *I. viscosa* to be used as a source for fungicidal preparations against plant foliar diseases caused by fungal pathogens [61, 62].

Phytochemical analysis of Inula species also afforded germacranolides (**192-209**), dimeric sesquiterpene lactones (**210-222**), terpenoids other than sesquiterpene lactones

(**223-309**), flavonoids (**310-334**) and miscellaneous compounds comprising derivatives of chlorogenic acid and anthranilic acid and aliphatic compounds (**335-381**) [56]. In general chemical studies of different *Inula spp*. have revealed the isolation of more than 350 natural products, and compound names and Inula species from which they were isolated are presented in Table 3 and corresponding structures in Fig. 4-12 [54, 56].

Table 3. List of secondary metabolites isolated from Inula species

Compound class and name [cpd no]	**Source Inula species**	**Ref.**
Eudesmanolides		
Alantolactone (**20**)	*I. helenium, I. racemosa,I. royleana,I. japonica, I. falconeri*	56, 63, 64
Isoalantolactone (**21**)	*I. helenium,I. racemosa,I. royleana, I. japonica, I. salsoloides*	54, 56
5α,6α-epoxyalantolactone (**22**), 11α,13-Dihydroalantolactone (**23**),11α-Hydroxyeudesm-5-en-12,8β-olide (**24**), Macrophyllilactone E (**25**)	*I. helenium, I. racemosa*	56, 64
4-oxoeudesma-5,11-dien-12,8β-olide (**26**),4-oxoeudesm-11-en-12,8β-olide (**27**), Dugesialactone [**28**],Alloantolactone (**29**), Telekin[**30**],4α,5α-epoxy alantolactone (**31**), 4α,15-Epoxyiso alantolactone (**32**), 3α-Hydroxyeudesma-4,11-dien-12,8β-olide (**33**), 11,13-Dihydro-3α-hydroxyalanto lactone (**34**), 15-Hydroxy-11βH-eudesm-4-en-12,8β-olide (**35**), 2β,11α-Dihydroxyeudesm-5-en-12,8β-olide (**36**), 11,13-Dihydro-1α-hydroxyisoalantolactone (**37**), 11α,13-Dihydro-β-cyclocostunilide (**38**), 11α,13-Dihydro-α-cyclocostunilide (**39**), Igalane (**40**), Umbellifolide (**41**)	*I. helenium*	54, 56
Pulchellin E (**42**)	*I. oculus-christi*	
1β-hydroxyalantolactone (**43**), Britannilactone (**44**), 1-O-acetylbritanni lactone (**45**), 1,6-Di-O-acetylbritannilactone (**46**)	*I. japonica,I. Britannica*	
8-epiivangustin (**47**), Ivalin (**48**) 6β-O-(2-Methyl butyryl)britannilactone (**49**), Neobritannilactone A (**50**)	*I. Britannica*	
Ivangustin (**51**), 1β,3α-ihydroxyeudesma-4,11(13)-dien-12,8β-olide (**52**), 1β,3β-Dihydroxyeudesma-4,11(13)-dien-12,8β-olide (**53**), 1β,2β-Dihydroxyeudesma-4,11(13)-dien-12,8β-olide (**54**), 1β-Hydroxy-3-oxoeudesma-4,11(13)-dien-12,8β-olide (**55**), 1β,4β Dihydroxyeude sma-5,11(13)-dien-12,8β-olide (**56**), 1β,3β-Dihydroxy-4αH-eudesma-5,11(13)-dien-12,8β-olide (**57**), Isoivasperin (**58**), 1β,5α-Dihydroxy-4αH-eudesm-11(13)-en-12,8β-olide (**59**), 5α-Hydroxyasperilin (**60**), 1β,3β-Dihydroxy-4αH-eudesma-5,11(13)-dieno-12,8β-lactone (**61**), 5α-Hydroperoxyasperilin (**62**),8β-Hydroxysantamarin (**63**), 11α,13-Dihydro-1β-hydroxyalantolactone (**64**),1β,5α-Dihydroxy-4αH,11αH-eudesman-12,8β -	*I. japonica*	

olide (**65**), 1β,5α-Dihydroxy-11αH-eudesm-4(15)-en-12,8β-olide (**66**), 1β,3α-Dihydroxy-11αH-eudesm-4-en-12,8β-olide (**67**), 5α-Hydroperoxy-1β-hydroxy-11αH-eudesm-4(15)-eno-12,8β-lactone (**68**), 1β,3β-Dihydroxy-4αH,11αH-eudesm-5-eno-12,8β-lactone (**69**), 1,10β-Dihydroxy-4αH-1,10-secoeudesma-5,11(13)-dien-12,8β-olide (**70**), 6α-(Acetyloxy)-1-hydroxy-4αH-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide (**71**), 1-Hydroxy-6α-(isobutyryloxy)-4αH-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide (**72**), 1-Hydroxy-6α-(2-methylbutyryloxy)-4αH-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide (**73**), 1-Hydroxy-6α-(isovaleryloxy)-4αH-1,10-seco-eudesma-5(10),11(13)-dien-12,8β-olide (**74**), 1,14-Dihydroxy-4αH-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide (**75**), 1-(Acetyloxy)-6α,14-dihydroxy-4αH-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide (**76**)		
Granilin(**77**),1α-Hydroxy-3α-senecioyloxy) isoalantolactone (**78**),1α-Hydroxy-3α-(isobutylryloxy) isoalantolactone (**79**), 1β-Hydroxy-11αH-eudesman-12,8α-olide (**80**)	*I. falconeri*	
1-Oxo-4-epialantolactone (**81**), Septuplinolide (**82**), 3-Oxoeudesma-4,11-dien-12,8β-olide (**83**), Isoalanto diene (**84**), 2α-Hydroxyeudesma-4,11(13)-dien-12,8β-olide (**85**), Dehydroivangustin (**86**), 11α,13-Dihydro-2α-hydroxyalantolactone (**87**), 11α,13-Dihydroivalin (**88**), 11α,13-Dihydro-3β-hydroxyalanto lactone (**89**), 13-(Acetyloxy)eudesma-5,7(11)-dien-12,8α-olide (**90**), 13-Hydroxyeudesma-5,7(11)-dien-12,8β-olide (**91**), 4α,13-Dihydroxy eudesma-5,7(11)-dien-12,8α-olide (**92**), 13-Hydroxy-4αH-eudesma-5,7(11)-dien-12,8α-olide (**93**), 13,13-Dimethoxy-4αH-eudesma-5,7(11)-dien-12,8β-olide (**94**)	*I. racemosa*	
4α,6α-Dihydroxyeudesma-2,11(13)-dien-12,8β-olide (**95**), 6α-Hydroxy-3-oxoeude sma-4,11(13)-dien-12,8β-olide (**96**), 6α-Hydroxyisoalloalantolactone (**97**), 6α-Hydroxy-4-episeptuplinolide (**98**), 6α-Hydroxyisoalantolactone (**99**), Arglanin (**100**),4α,6α-Dihydroxy-11αH-eudesman-12,8β-olide (**101**), Tauremisin (**102**), 6α-Hydroxy-2,3-secoeudesma-1,3,11(13)-trien-12,8β-olide (**103**)	*I. hupehensis*	
11α,13-Dihydroisoalantolactone (**104**)	*I. helenium,I. japonica*	
1,6α-Dihydroxyeriolanolide (**105**), 1-Acetoxy-6α-hydroxyeriolanolide (**106**)	*I. japonica,I. britannica var. chinensis*	
Guaianolides		
Inuviscolide (**107**)	*I. hupehensis, I. falconeri, I. racemosa,I. hookeri,I. cappa, I. viscosa*	
Dehydrocostuslactone (**108**)	*I. helenium*	
Gaillardin (**109**)	*I. oculus-christi,I. linariifolia, I. hookeri*	
4-Epiisoinuviscolide (**110**)	*I. linariifolia, I. falconeri, I. hupehensis, I. hookeri, I. graveolens*	

Xerantholide (**111**)	*I. linariifolia, I. falconeri*	54, 56
Britanlin D (**112**)	*I. britannica*	
2α-(Acetyloxy)-5α,6α-epoxy-4α-hydroxy-1βH-guai-11(13)-en-12,8α-olide (**113**), 2α-(Acetyloxy)-4α-hydroxy-1βH-guaia-10(14),11(13)-dien-12,8α-olide (**114**)	*I. linariifolia, I. hookeri*	
8-Epiinuviscolide (**115**)	*I. hupehensis, I. falconeri,I. helianthus aquatilis, I. sericophylla, I. hookeri*	
Inuchinenolide B (**116**)	*I.hupehensis,I. hookeri*	
6α-(Acetyloxy)isoinuviscolide (**117**)	*I. hupehensis,I. sericophylla*	
2α-(Acetyloxy)-4α,6α-dihydroxy-1βH-guaia-9,11(13)-dien-12,8α-olide (**118**), 4β,10α-Dihydroxyguaia-1,11(13)-dien-12,8α-olide (**119**)	*I. hupehensis*	
11β,13-Dihydroinuviscolide (**120**), 11α,13-Dihydroinuviscolide (**121**)	*I. falconeri, I. hookeri*	
4α,5α-Epoxy-10α,14-dihydro-1-epiinuviscolide (**122**), 4,8-Bisepiinuviscolide (**123**), 6α-Hydroxyinuviscolide (**124**), 4α,6α-Dihydroxy-1βH-guaia-9,11(13)-dien-12,8α-olide (**125**), Postia secoguaianolide (**126**)	*I. falconeri*	
14-(Acetyloxy)-4β-hydroxy-1βH-guaia-9,11(13)-dien-12,8α-olide (**127**)	*I. hookeri*	
Florilenalin (**128**)	*I. salsoloides*	
Pseudoguanianolides		
Ergolide (**129**)	*I. britannica,I. hupehensis, I. falconeri,I. sericophylla, I. hookeri,I. helianthus*	
8-Epihelenalin (**130**), Graveolide or 2,3-dihydroaromaticin (**131**)	*I. graveolens,I. hupehensis, I. sericophylla,I. hookeri,I. falconeri*	
Bigelovin (**132**)	*I. graveolens,I. hupehensis, I. I. hookeri, I. helianthus aquatilis, I. britannica var.chinensis*	
11α,13-Dihydroconfertin (**133**)	*I. graveolens*	
2-O-Acetyl-4-epipulchellin (**134**)	*I. linariifolia, I. britannica, I. helianthus aquatilis,*	
2-Desoxy-4-epipulchellin (**135**)	*I. linariifolia, I. britannica, I. helianthus aquatilis*	
2α,6α-Bis(acetyloxy)-4β-hydroxy-10βH-pseudoguai-11(13)-en-12,8α-olide (**136**)	*I. linariifolia*	
2α,4β-Dihydroxypseudoguai-11(13)-en-12,8α-olide (**137**), 2α-(Acetyloxy)-4β-hydroxypseudoguai-11(13)-en-12,8α-olide (**138**)	*I. japonica*	
Carpesiolin or 6α-hydroxy-2,3-dihydroaromaticin (**139**)	*I. hupehensis, I. falconeri, I. sericophylla, I. hookeri*	
Confertin (**140**), 8α-Hydroxy-4-oxo-10βH-pseudoguaia-2,11(13)-dien-12,6α-olide (**141**), 6α-Hydroxy-4-oxo-10βH,11αH-pseudoguai-2-en-12,8α-	*I. hupehensis*	

olide (**142**), 14-(Acetyloxy)-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**143**), 6α-Hydroxy-2β-methoxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**144**), 2β-Ethoxy-6α-hydroxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**145**), 6α-(Acetyloxy)-2β-methoxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**146**), 6α-(Acetyloxy)-2α-methoxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**147**), 6α-Hydroxy-4-oxo-10βH,11βH-pseudoguai-12,8α-olide (**148**)		54, 56
Burrodin (**149**)	*I. hupehensis, I. hookeri*	
Aromaticin (**150**)	*I. hupehensis, I. hookeri, I. sericophylla,*	
11β,13-Dihydroergolide (**151**)	*I. falconeri, I. sericophylla, I. helianthus aquatilis*	54, 56
11β,13-Dihydrocarpesiolin (**152**)	*I. falconeri*	
2,3,11β,13-Tetrahydroaromaticin (**153**), 6α-(Acetyloxy)-4β-hydroxy-10βH-pseudoguai-11(13)-en-12,8α-olide (**154**),4β-(Acetyloxy)-6α-hydroxy-10βH-pseudoguai-11(13)-en-12,8α-olide (**155**)	*I. falconeri , I. helianthus aquatilis*	
10α-Hydroxy-8-epiconfertin (**156**), 6α-(Acetyloxy)-9β-hydroxy-4-oxo-10βH,11αH-pseudoguai-2-en-12,8α-olide (**157**), 6α-(Acetyloxy)-2β-ethoxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**158**), 6α-(Acetyloxy)-2β-hydroxy-4-oxo-10βH-pseudoguai-11(13)-en-12,8α-olide (**159**)	*I. hookeri*	
4-Epipulchellin (**160**), Britanlin I (**161**), Britanlin J (**162**), Inuchinenolide C (**163**), Britanin (**164**), Deacetylbritanin (**165**), 2-O-Acetyl-11β,13-dihydro-4-epipulchellin (**166**)	*I. Britannica*	
Xanthanolides		
Tomentosin (**167**)	*I. viscosa, I. graveolens,I. linariifolia,I. japonica,I. britannica, I .falconeri, I. sericophylla, I. hookeri, I. hupehensis*	
(3αR,7S,8αR)-3,3α,4,7,8,8α-Hexahydro-6-(3-hydroxybutyl)-7-methyl-3-methylidene-2H-cyclohepta[b]furan-2-one (**168**)	*I. graveolens, I. japonica, I. royleana, I. falconeri, I. sericophylla, I. hookeri*	
2α-Hydroxytomentosin (**169**), 4,5-Dioxo-11βH-xanth-1(10)-en-12,8β-olide (**170**)	*I. graveolens*	
11α,13-Dihydrotomentosin (**171**)	*I. falconeri,I. sericophylla, I. hupehensis,I. hookeri*	
(3S,3αR,7S,8αR)-3,3α,4,7,8,8α-Hexahydro-6-(3-hydroxybutyl)-3,7-dimethyl-2H-cyclohepta[b]furan-2-one (**172**)	*I. falconeri, I. sericophylla*	
(3R,3αR,7S,8αR)-3,3α,4,7,8,8α-Hexahydro-6-(3-hydroxybutyl)-3,7-dimethyl-2H-cyclohepta[b]furan-2-one (**173**)	*I. sericophylla,I. hookeri*	
Carabrone or grandicin (**174**)	*I. viscosa, I. falconeri, I. cappa, I. hookeri, I. royleana, I. hupehensis,I.*	54, 56

	helenium	
8-Epixanthatin-1b,5β-epoxide (**175**)	*I. graveolens, I. hookeri, I. hupehensis, I. Britannica*	
Inuchinenolide A (**176**)	*I. linariifolia, I. hupehensis*	
Ivalbatin (**177**)	*I. viscosa, I. helenium*	
Sundiversifolide (**178**)	*I. sericophylla,I. hupehensis, I. hookeri*	54, 56
11-Episundiversifolide (**179**)	*I. hupehensis,I. hookeri*	
6β-Hydroxytomentosin (**180**)	*I. linariifolia, I. britannica, I. hupehensis*	
(3αR,7R,8αR)-3,3α,4,7,8,8α-Hexahydro-6-(3-hydroxybutyl)-7-methyl-3-methylidene-2H-cyclohepta [b]furan-2-one (**181**), 4-[(3αR,7S,8αR)-3,3α,4,7,8,8α-Hexahydro-7-methyl-3-methylidene-2-oxo-2H-cyclo hepta[b]furan-6-yl]butan-2-yl acetate (**182**), 11β,13-Dihydro-6α-hydroxytomentosin (**183**), 1β,5β-Epoxy-4-hydroxy-10αH-xanth-11(13)-en-12,8β-olide (**184**)	*I. falconeri*	
6α-Hydroxytomentosin (**185**), 6α-Hydroxycarabrone (**186**), 14-(Acetyloxy)-6α-hydroxycarabrone (**187**)	*I. hupehensis*	
Britanlin F (**188**), Britanlin H (**189**)	*I. Britannica*	
Britanlin G (**190**)	*I. britannica, I. sericophylla*	
8β-(Propionyloxy)inusoniolide (**191**)	*I. linariifolia*	
Germacranolides		
Eupatolide (**192**), Neobritannilactone B (**193**), Acetyl neobritannilactone B (**194**)	*I. britannica var. chinensis, I. japonica, I. salsoloides*	54, 56
4β,5α-Epoxygermacra-1(10),11(13)-dien-12,8α-olide (**195**), Isocostunolide (**196**), Costunolide (**197**), 11β,13-Dihydro-14-hydroxycostunolide (**198**), (1(10)E)-5β-Hydroxygermacra-1(10),4(15),11 (13)-trien-12,8α-olide (**199**)	*I. helenium*	
Inulasalsolide (**200**), 4α,5β-Epoxyeupatolide (**201**), Inulasalsolide B (**202**)	*I. salsoloides*	
1β,10α,4α,5β-Diepoxy-7βH-germacr-11(13)-en-12,8β-olide (**203**)	*I. falconeri*	
Ineupatolide A (**204**), Inulacappolide (**205**)	*I. cappa*	
9β-Hydroxyeupatolide (**206**), 9β-(Acetyloxy) eupatolide (**207**), 8β-Acetoxy-1β,10α-epoxy-9β-hydroxyeupatolide (**208**)	*I. hupehensis*	
11,13-Dehydroivaxillin (**209**)	*I. hupehensis,I. hookeri*	
Dimeric sesquiterpenes		
Inulanolide A ,C, D (**210-212**)	*I. britannica var chinensis*	54, 56
Japonicone A-D (**213-216**), Japonicone H (**217**) Japonicone E-G (**218-220**), Japonicone J (**221**)	*I. japonica*	
Bialantolactone (**222**)	*I. helenium*	
Other terpenenoids		
9-Hydroxythymol 9-O-β-d-glucopyranoside (**223**)	*I. hupehensis*	
8,9,10-Trihydroxythymol (**224**), 8-Hydroxy-9,10-bis(isobutyryloxy)thymol (**225**)	*I. hupehensis,I. salsoloides, I. japonica,*	

	I. sericophylla, I. hupehensis, I. japonica	
8,9-Epoxy-10-(isobutyryloxy)thymol isobutyrate (**226**)	*I. hupehensis,I. royleana, I. helenium, I. ensifolia, I. sericophylla*	
8,9-Epoxy-10-(isobutyryloxy)-6-methoxy thymol isobutyrate (**227**)	*I. royleana*	
8,9-Epoxy-10-(2-methylbutyryloxy)thymol isobutyrate (**228**)	*I. helenium,I. sericophylla,I. ensifolia*	
7-(Isobutyryloxy)thymol methyl ether (**229**), 8,9-Epoxy-10-(isovaleryloxy)thymol isobutyrate (**230**), 8,9-Epoxy-3-(isobutyryl)-7-(isobutyryl oxy)thymol-10-yl-2-methylbutanoate (**231**), 5β,6β-Epoxy-3b-hydroxy-β-ionone (**232**)	*I. ensifolia*	
Thymol (**233**), Thymol isobutyrate (**234**), Thymol isovalerate (**235**), 8α-Hydroxypresilpheperfolene (**236**), Intermedeol (**237**)	*I. cuspidate*	
Isothymol (**238**), Zataroside (**239**), (Z)-Abienol (**240**), Epifriedelanol (**241**), α-Amyrin (**242**), β-Amyrin (**243**), Oleanolic acid (**244**), Dammara-20,24-dien-3-yl acetate (**245**), Dammara-20,24-dien-3β-ol (**246**), 7-Oxo-β-sitosterol (**247**), Stigmast-4-en-3-one (**248**), Stigmasta-4,22-dien-3-one (**249**),Stigmast-5-ene-3β,7β-diol (**250**), Stigmasta -5,22-diene-3β,7β-diol (**251**), Stigmast-5-ene-3β,7α-diol (**252**), Stigmasta-5,22-diene-3β,7α-diol (**253**)	*I. cappa*	54, 56
9,10-Dihydroxythymol (**254**), Inulasalene (**255**)	*I. salsoloides*	
8,9-Epoxy-9,10-dihydroxythymol (**256**), Grasshopper ketone (**257**), Corchoinol (**258**), Vomifoliol (**259**), 8β-(Acetyloxy)-1β,4α-dihydroxyeudesm-11(13)-en-12-oic acid methyl ester (**260**), 8β-(Acetyloxy)-1β-hydroxyisocostic acid methyl ester (**261**), 8β-(Acetyloxy)-1β-hydroxycostic acid methyl ester (**262**), 16αH-*ent*-Kauran-19-oic acid 17-O-β-d-glucopyranoside (**263**), 17-Hydroxy-16αH-*ent*-kauran-19-oic acid (**264**), 16α,17-Dihydroxy-ent-kauran-19-oic acid (**265**), 16α-Hydroxy-17-(acetyloxy)-*ent*-kauran-19-oic acid (**266**)	*I. japonica*	
8,10-Dihydroxy-9-(isobutyryloxy)thymol (**267**), 8-Hydroxy-9-(isobutyryloxy)-10-(2-methyl butanoyl)thymol (**268**)	*I. japonica, I. sericophylla*	
7-Epiloliolide (**269**)	*I. japonica,I. helianthus-quatilis*	
Loliolide (**270**)	*I. helianthus-aquatilis,I. ensifolia, I. sericophylla*	
11,12,13-Trinor-4αH-eudesm-5-ene-7β,8α-diol (**271**), Racemosin A (**272**), 4β-Eudesm-11(13)-ene-4α,12-diol (**273**), 8β-Hydroxyeudesma-4,11(13)-dien-12-oic acid (**274**)	*I. racemosa*	
Trinoralantolactone (**275**), (1(10)*Z*)-4,5-Dioxo-4,5-seco-7βH-guaia-1(10),11-diene (**276**), Dammarane-3,20-diol (**277**), 20-Hydroxy dammaran-3-yl acetate (**278**)	*I. helenium*	
Costic acid (**279**), Isocostic acid (**280**)	*I. graveolens, I. viscosa*	

2α-Hydroxyisocostic acid (**281**), 3α-Hydroxy costic acid (**282**), Ilicic acid (**283**), 3α-Hydroxy ilicic acid (**284**), 4-Epiilicic acid (**285**), 3α-Hydroxy-4-epiilicic acid (**286**), 3α-Hydroxyilicic acid methyl ester (**287**), 2α-Hydroxy-4-epiilicic acid (**288**)	*I. graveolens*	
Liguloxidol (**289**), Britanlin A (**290**), Lupeol (**291**)	*I. Britannica*	
Britanlin C (**292**)	*I. britannica,I. linariifolia*	
2β,5α-Dihydroxyisocostic acid (**293**), 2β,3α-Dihydroxycostic acid (**294**)	*I. viscosa*	
Ineariifolianone (**295**)	*I. linariifolia*	
Viridiflorol (**296**)	*I. oculus-christi*	
5-(Linoleoyloxy)-4,5-secocaryophyllen-4-one (**297**)	*I. falconeri*	54, 56
(13*E*)-Neocleroda-3,13-diene-15,18-diol (**298**), Vanclevic acid B (**299**), Vanclevic acid B methyl ester (**300**), 14,15-Dehydro-18,19-dihydroxyphytol (**301**), 19-[(3-Carboxy propanoyl)oxy]-14,15-dehydro-18-hydroxyphytol (**302**), 15,16-Dehydro-14,18,19-trihydroxyphytol (**303**)	*I. nervosa*	
Friedelin (**304**)	*I. cappa, I. helenium*	
Ursolic acid (**305**)	*I. cappa, I. helenium, I. japonica*	
β-Sitosterol (**306**)	*I. cappa, I. salsoloides,I. helenium, I. japonica*	
Stigmasterol (**307**)	*I. cappa, I. salsoloides,I. helenium, I. helianthus-aquatilis*	54, 56, 65
Daucosterol (**308**)	*I. cappa, I. japonica, I. helianthus-aquatilis*	
Stigmasterol 3-O-β-d-glucopyranoside (**309**)	*I. japonica, I. helenium*	
Flavonoids		
Japonicin A (**310**), Japonicin B (**311**), Onopordin (**312**), Nepetin (**313**), Patuletin (**314**), Nepitrin (**315**), 3'-O-Methylorobol (**316**)	*I. japonica*	
Apigenin (**317**)	*I. japonica, I. cappa,I. salsoloides*	
Luteolin (**318**)	*I. japonica, I. britannica,I. salsoloides*	
Quercetin (**319**)	*I. japonica, I. britannica,I. helenium*	
Isoquercitrin (**320**)	*I. japonica, I. ensifolia,I. helenium*	
6-Methoxyluteolin (**321**), 6-Methoxyluteolin (**322**), Isorhamnetin (**323**)	*I. Britannica*	
Sakuranetin (**324**), 7-O-Methylaromadendrin (**325**), 3-Acetyl-7-O-methylaromadendrin (**326**)	*I. viscosa*	54, 56
Hispidulin (**327**)	*I. oculus-christi*	
Luteolin 3'-O-b-d-glucopyranoside (**328**), 2-Hydnocarpin 7-O-b-d-glucopyranoside (**329**)	*I. cappa*	
Hyperin (**330**)	*I. ensifolia*	
Kaempferol (**331**), Acacetin (**332**), 4',7-Dihydroxyflavone (**333**), 3',4',7-Trihydroxy-5-methoxyflavone (**334**)	*I. salsoloides*	

Miscellaneous compounds		
Inuloxins A (**335**), Inuloxins B (**336**), Inuloxins C (**337**), Inuloxins D (**338**), 1,3-Di-O-caffeoylquinic acid (**339**)	*I. viscosa*	
Chlorogenic acid (**340**)	*I. cappa, I. viscosa,I. ensifolia*	
4-O-Caffeoylquinic acid (**341**), 1-O-Caffeoylquinic acid (**342**), 3-O-Caffeoylquinic acid (**343**)	*I. viscosa, I. helenium*	
3,4-Di-O-caffeoylquinic acid (**344**)	*I. viscosa, I. ensifolia*	
1,5-Di-O-caffeoylquinic acid (**345**)	*I. viscosa, I. ensifolia,I. crithmoides, I. britannica*	
3,5-Di-O-caffeoylquinic acid (**346**), Tocopherol (**347**)	*I. ensifolia*	66
Macranthoin F (**348**), Macranthoin G (**349**), (1β,2β,3β,4α,5β,6α)-Inositol 2,3,6-triangelate (**350**), l-Inositol 1,2,3,5-tetraangelate (**351**), Ceplignan (**352**), Protocatechualdehyde (**353**), Syringic acid (**354**), Sinapylaldehyde (**355**), Scopolin (**356**), 4-Allyl-2,6-dimethoxyphenol 1-O-β-d-glucopyranoside (**357**), Vanillic acid 4-O-β-d-glucopyranoside (**358**), Tachioside (**359**), Isotachioside (**360**), Syringic acid 4-O-α-l-rhamnoside (**361**)	*I. cappa*	
3,5-Di-O-caffeoylquinic acid 1-methyl ether (**362**), 4,5-Di-O-caffeoylquinic acid 1-methyl ether (**363**)	*I. crithmoides*	
(*Z*)-5-O-Caffeoylquinic acid (**364**), 5-O-Feruloyl quinic acid (**365**), (*Z*)-5-O-p-Coumaroylquinic acid (**366**), 5-O-p-Coumaroylquinic acid (**367**), 3-O-Caffeoyl-4-O-p-coumaroylquinic acid (**368**), Rosmarinic acid (**369**)	*I. helenium*	
Coniferyl diangelate (**370**), Sinapyl diangelate (**371**), Nervolan A (**372**), Nervolan B (**373**), Nervolan C (**374**), Inulavosin (**375**)	*I. nervosa*	
Dillapiole (**376**), Methyl eugenol (**377**)	*I. oculus-christi*	
N-Henicosanoylanthranilic acid (**378**), *N*-Tricosanoylanthranilic acid (**379**), *N*-Tetracosanoylanthranilic acid (**380**), *N*-Arachidylanthranilic acid (**381**), *N*-Docosanoylanthranilic acid (**382**)	*I. japonica*	
11-Hentriacontene (**383**), 17-pentatriacontene (**384**),pentatriacontane (**385**)	*I. granitites*	54, 56, 65

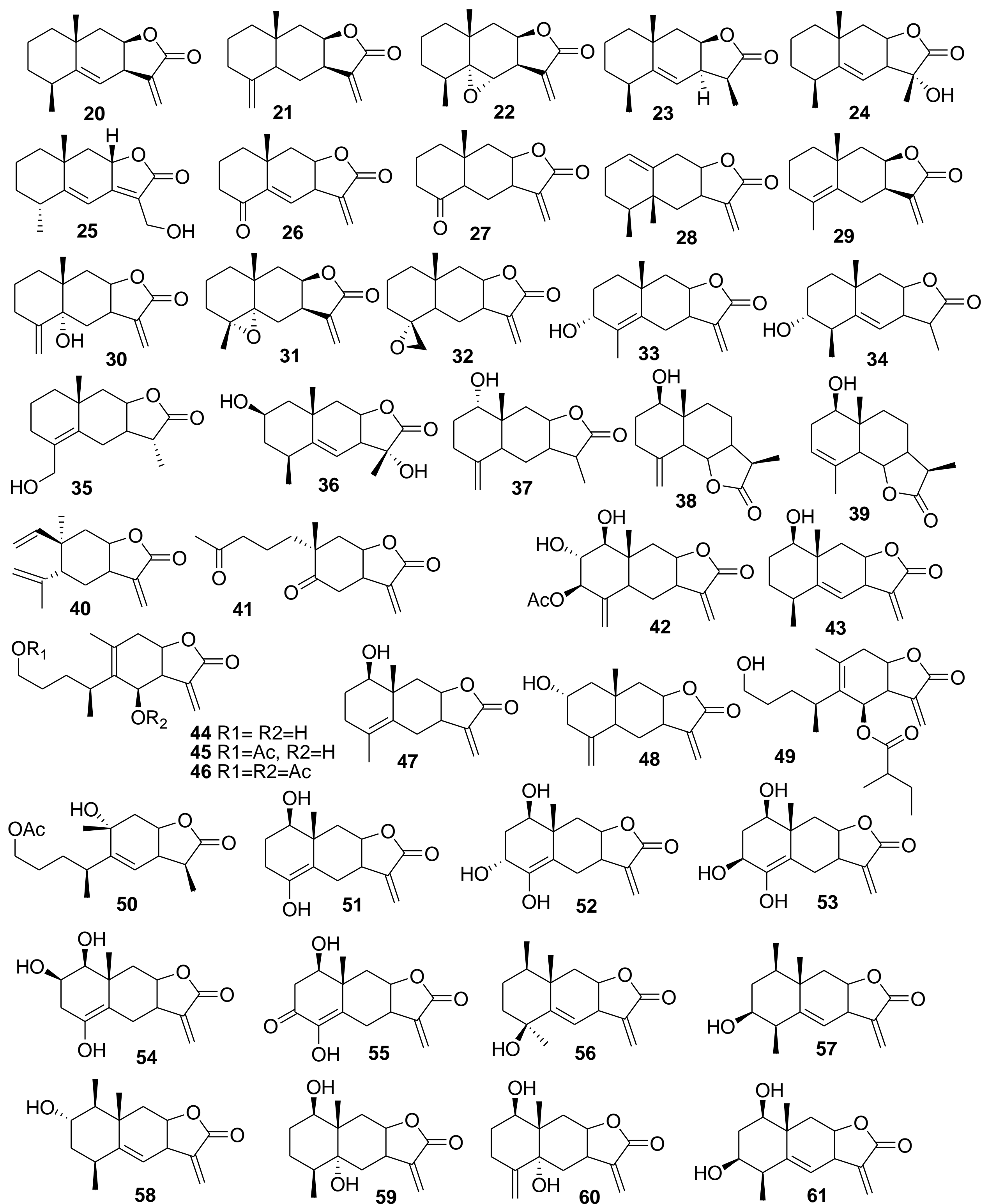


Fig. 4 Structures of eudesmanolides isolated from *Inula spp.*

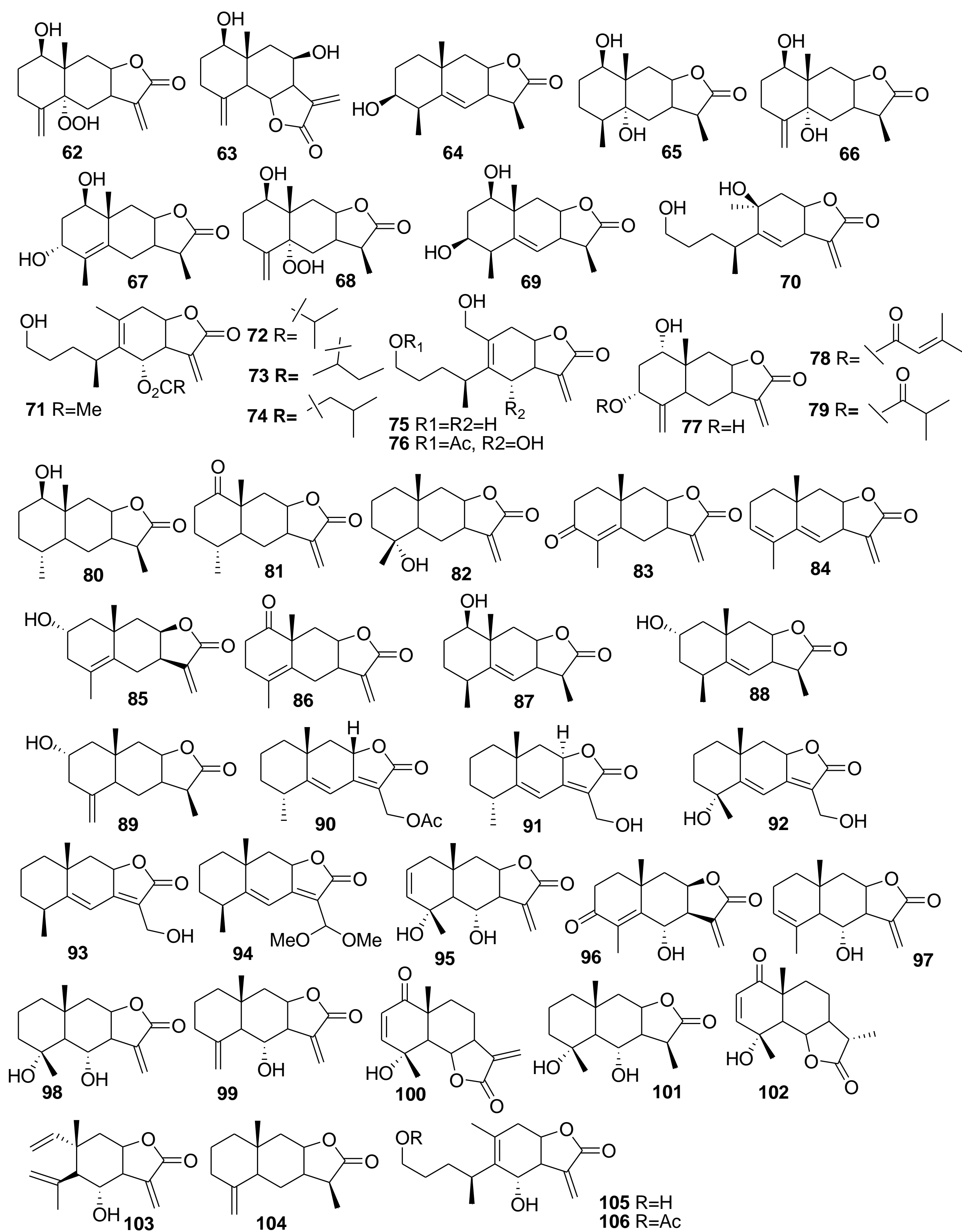


Fig. 4 Structures of eudesmanolides isolated from *Inula spp.* (cont.)

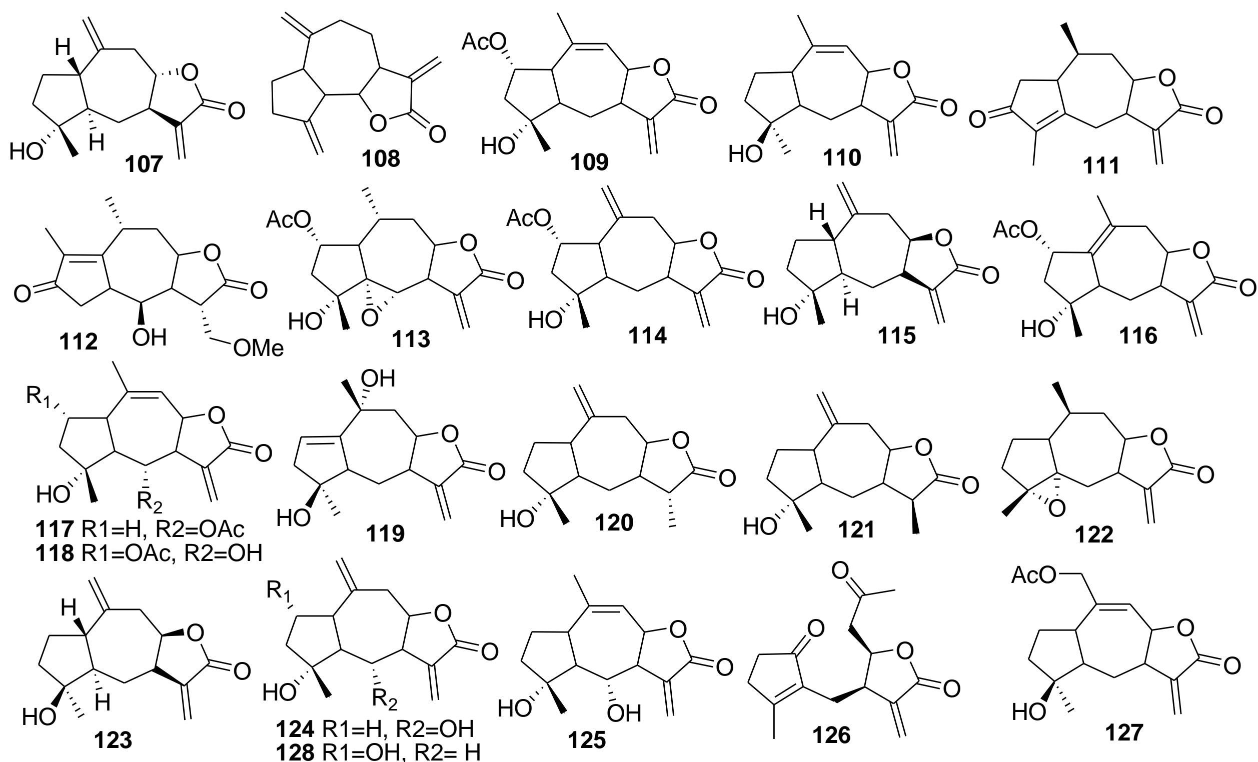


Fig. 5 Structures of guaianolides isolated from Inula spp.

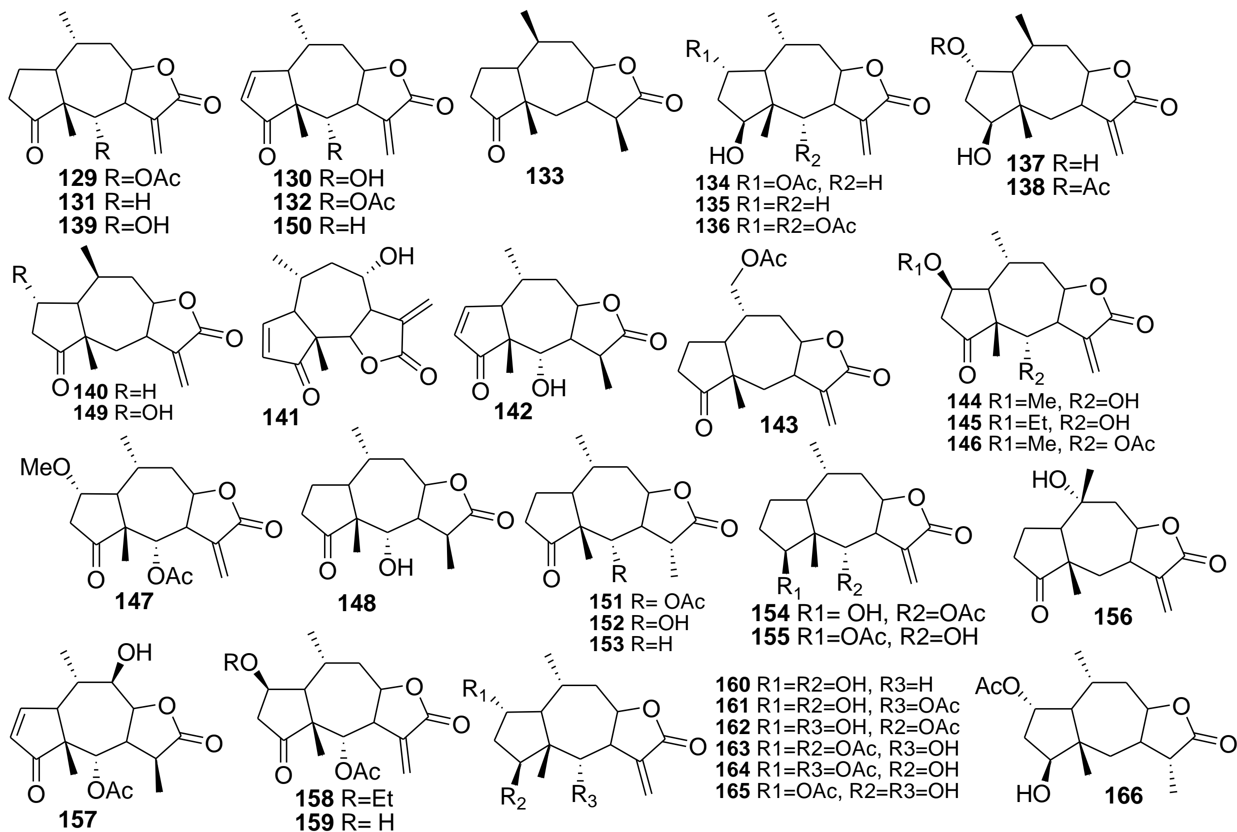


Fig. 6 Structures of pseudoguaianolides isolated from *Inula spp.*

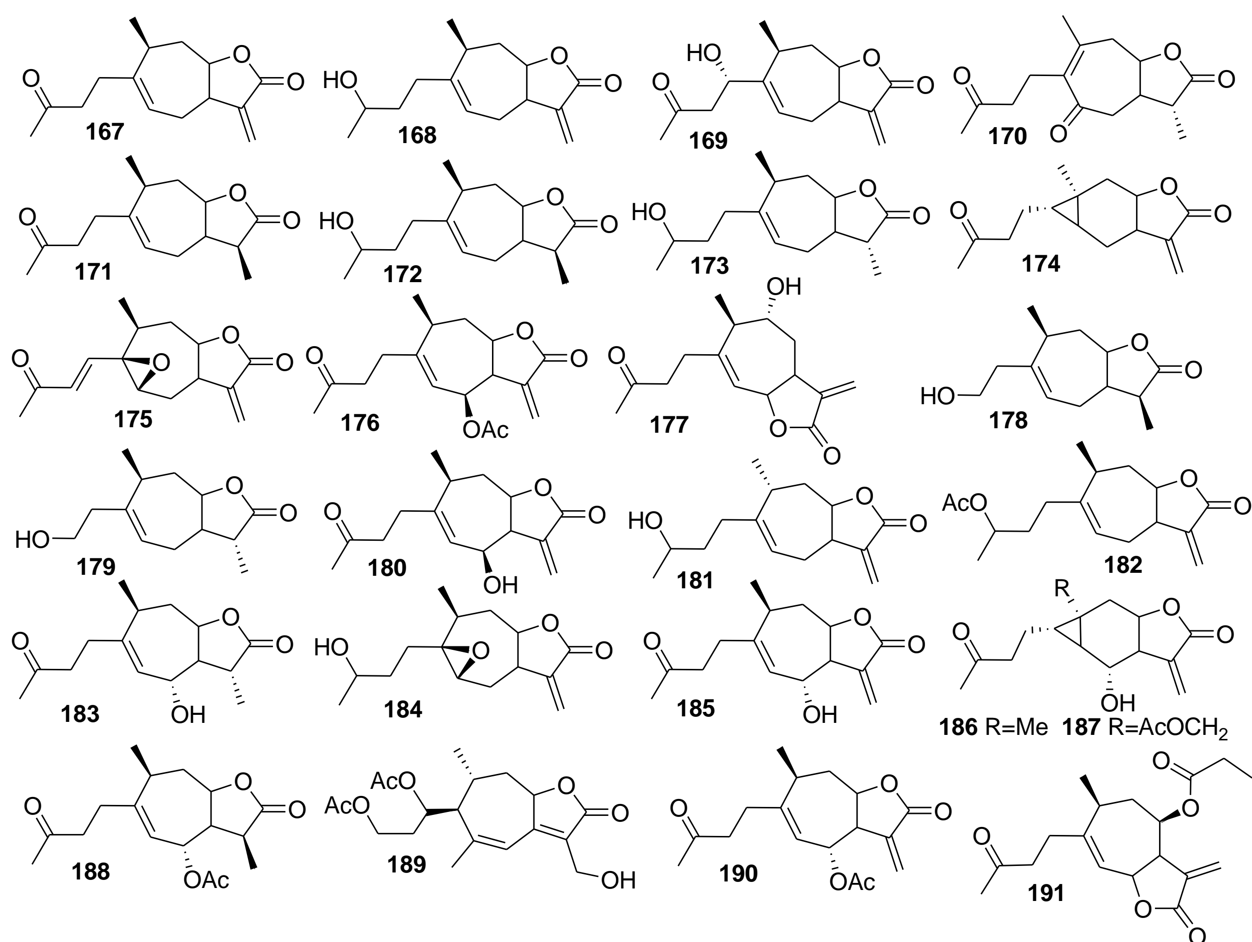


Fig. 7 Structures of xanthanolides isolated from *Inula spp.*

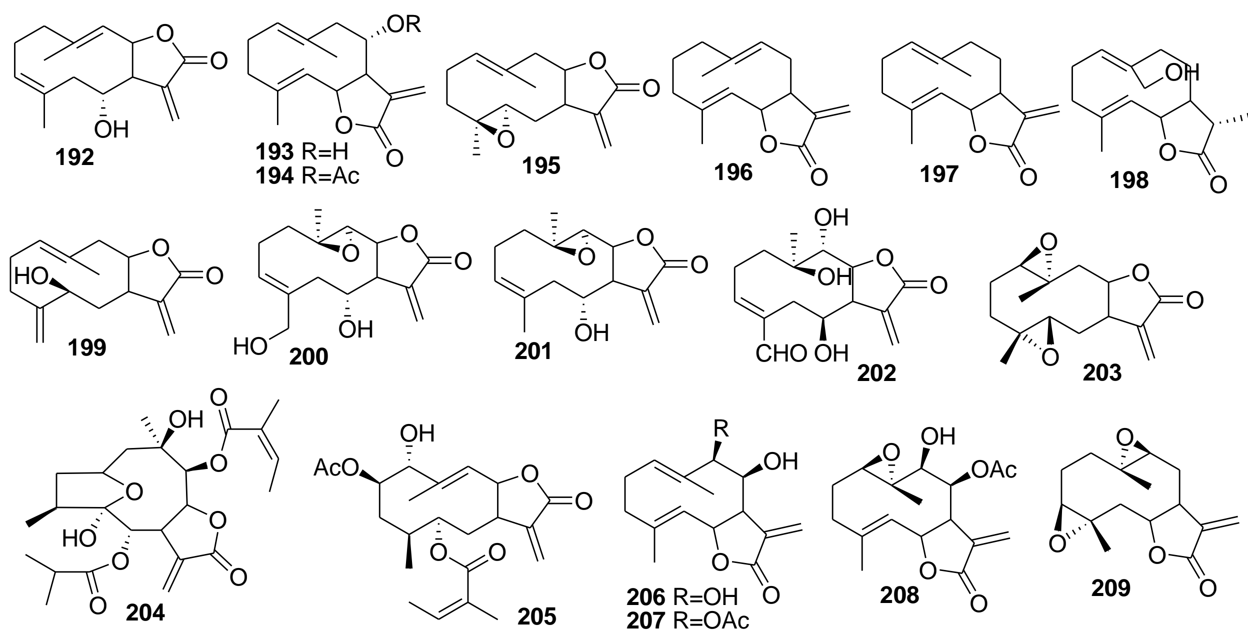


Fig. 8 Structures of germacranolides isolated from *Inula spp.*

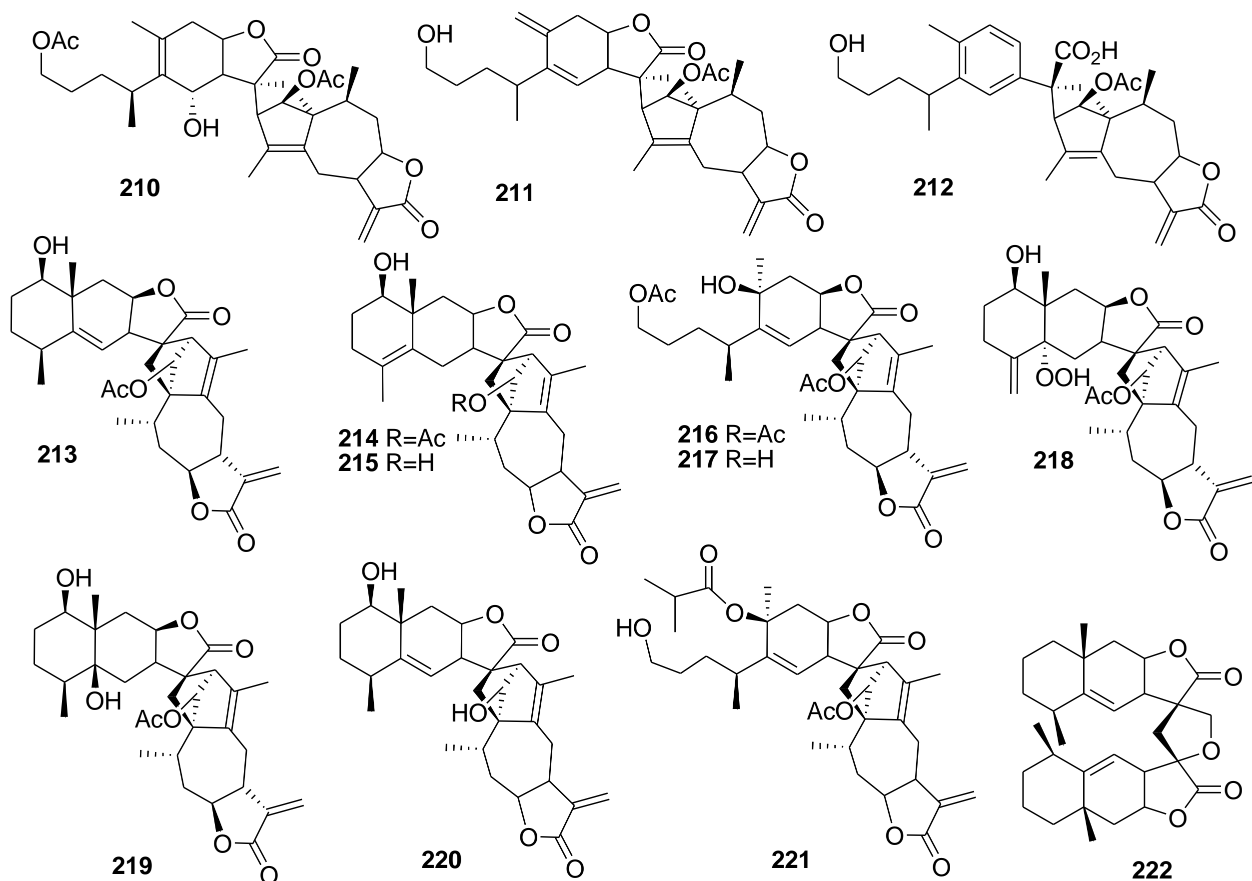


Fig. 9 Structures of dimeric sesquiterpene lactones isolated from Inula spp.

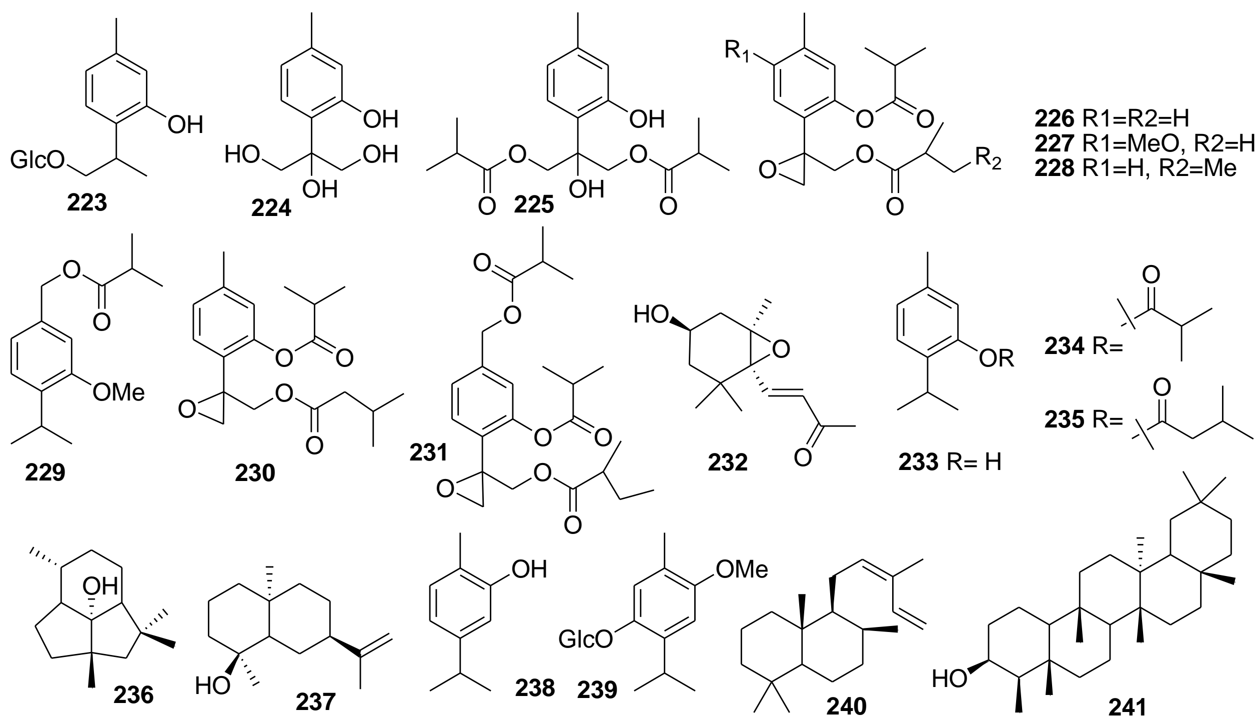


Fig. 10 Structures of other terpenoides isolated from Inula spp

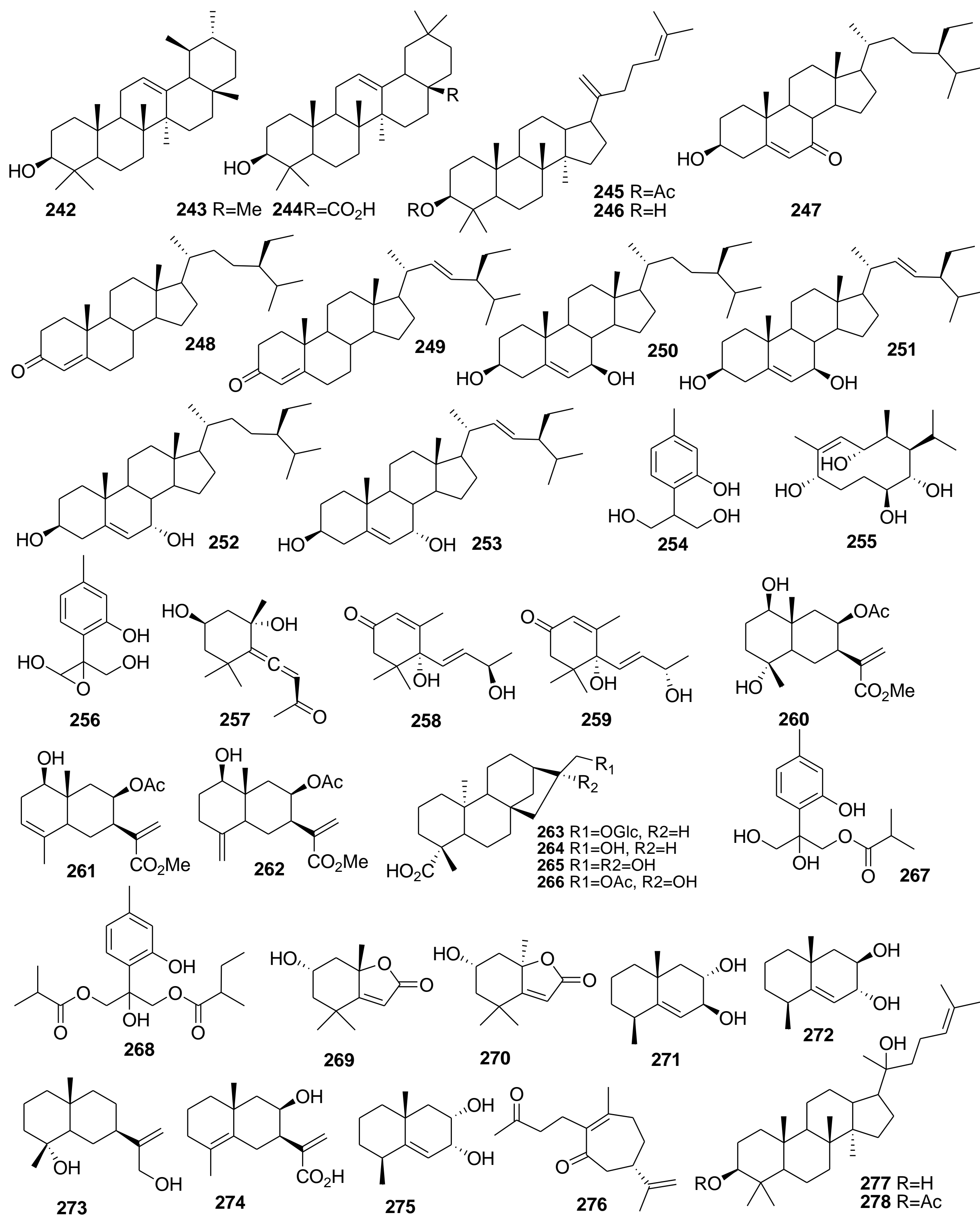


Fig. 10 Structures of other terpenoides isolated from *Inula spp.* (cont.)

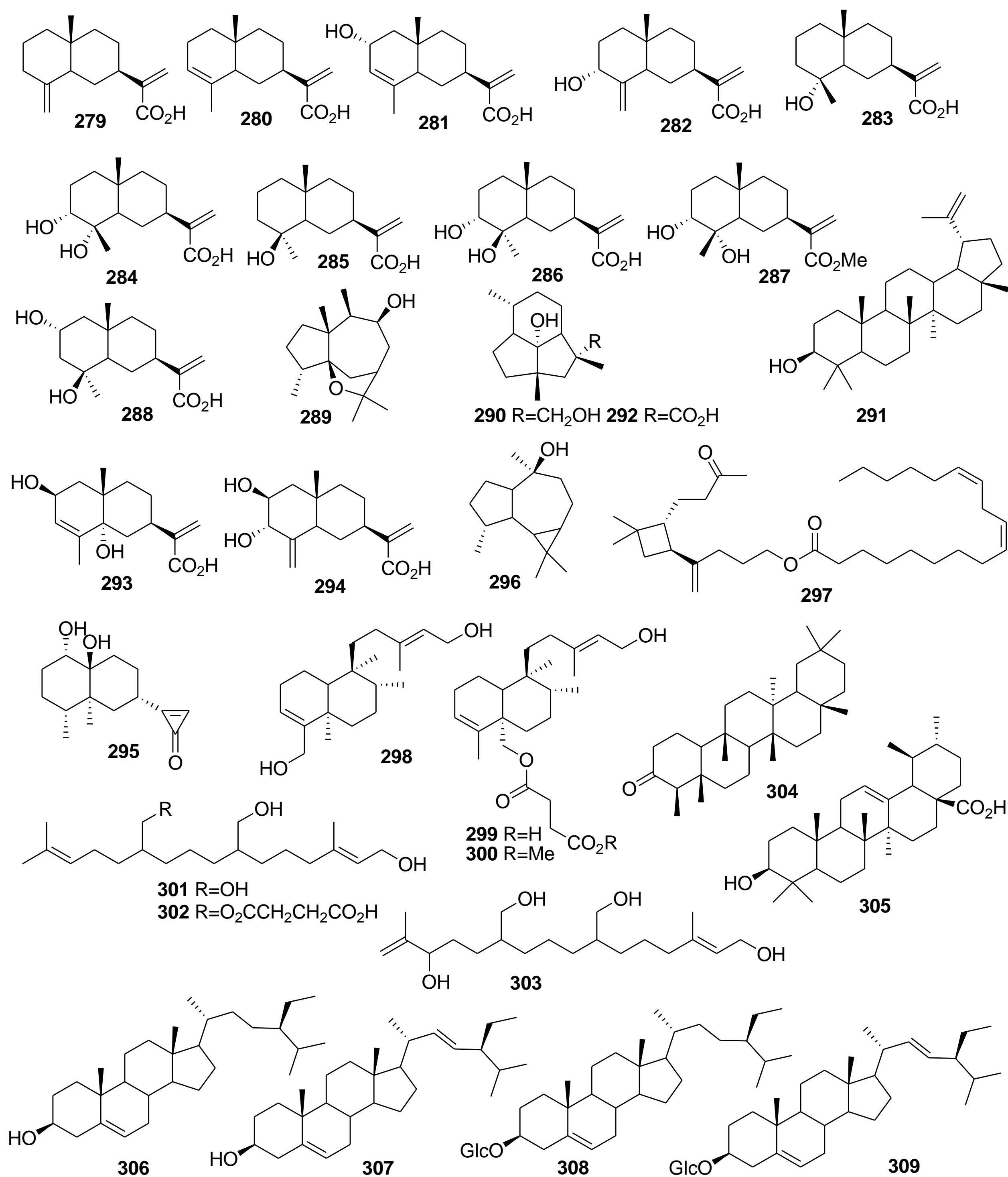


Fig. 10 Structures of other terpenoides isolated from *Inula spp.* (cont.)

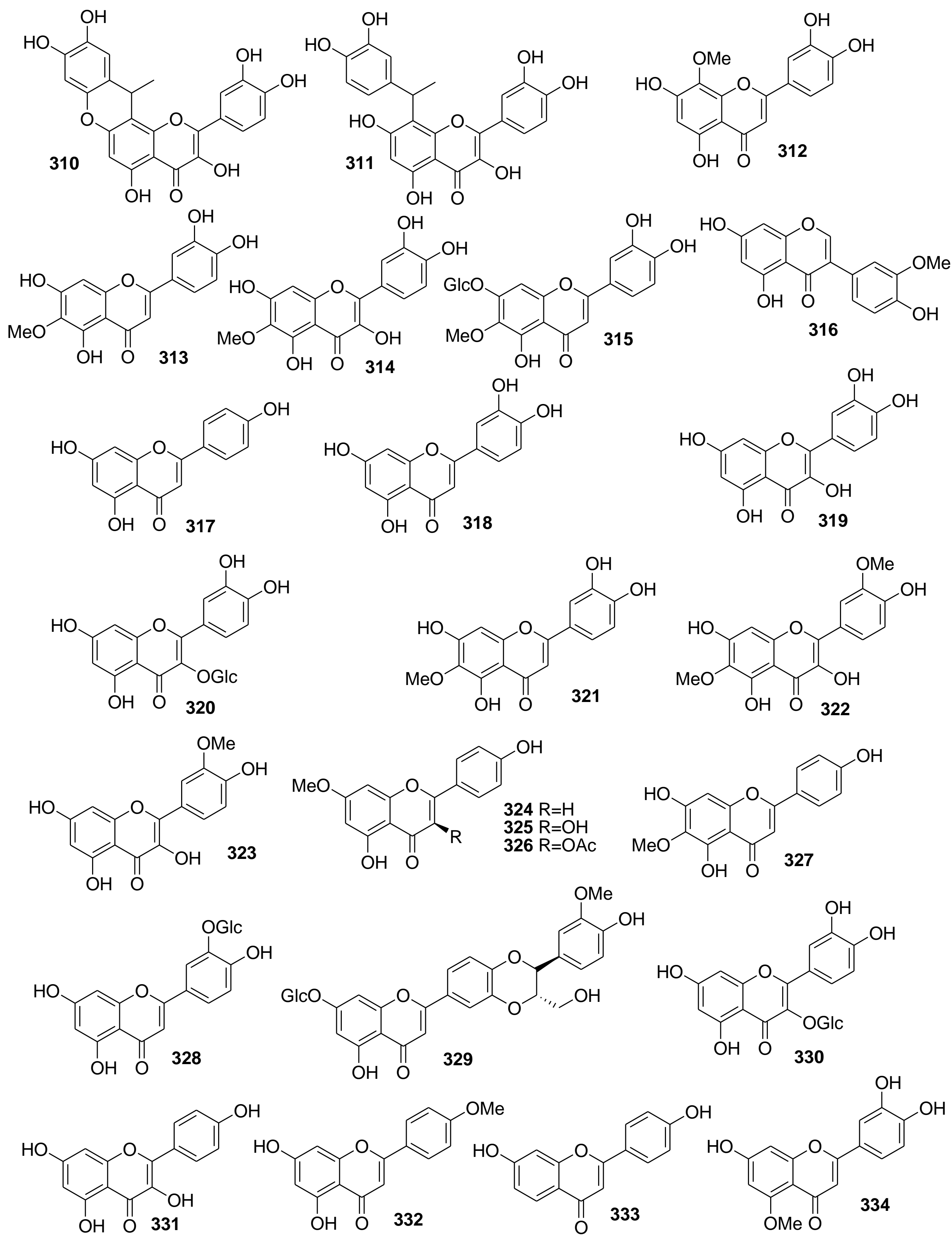


Fig. 11 Structures of flavonoids isolated from *Inula spp.*

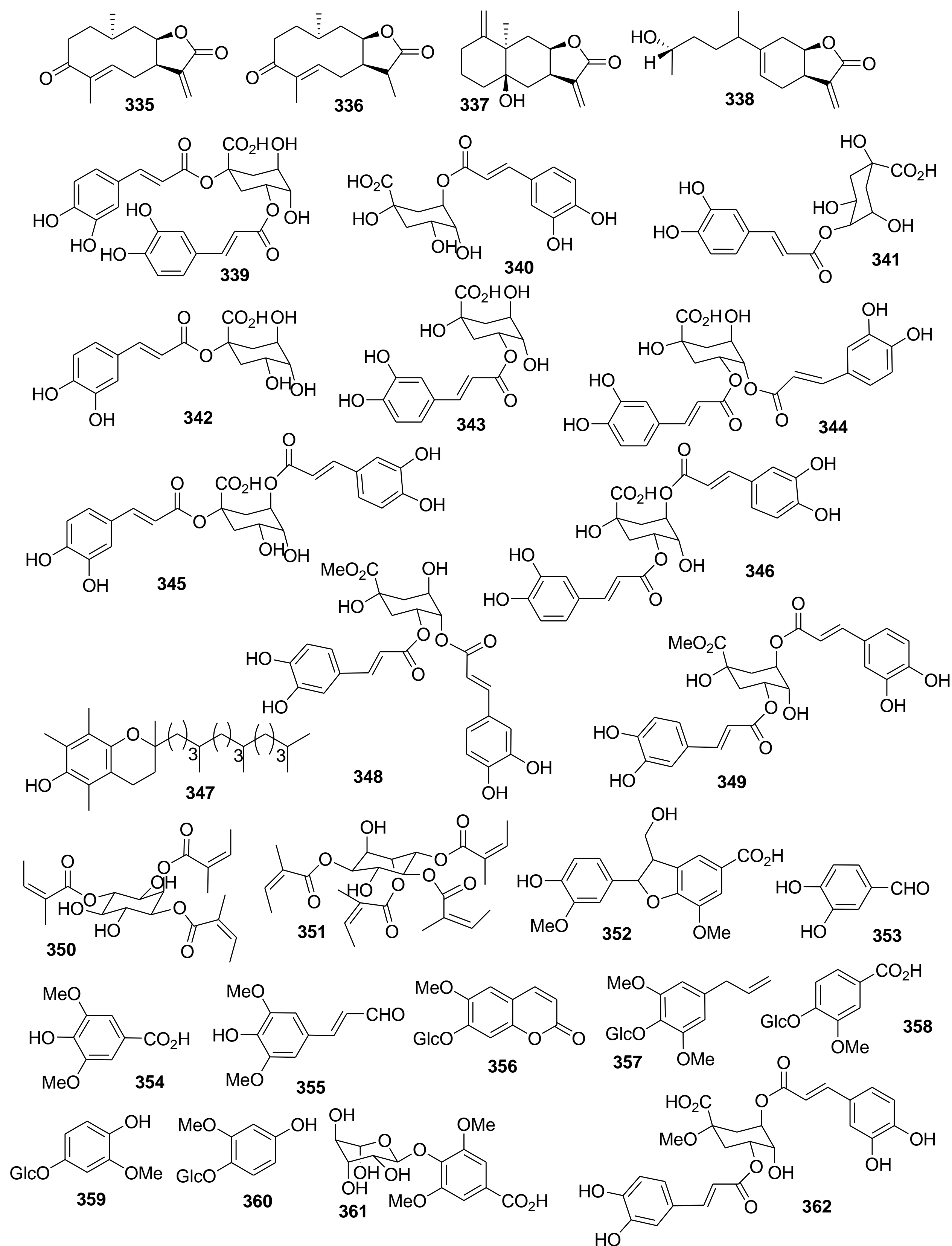


Fig. 12 Structures of miscellaneous compounds isolated from *Inula spp.*

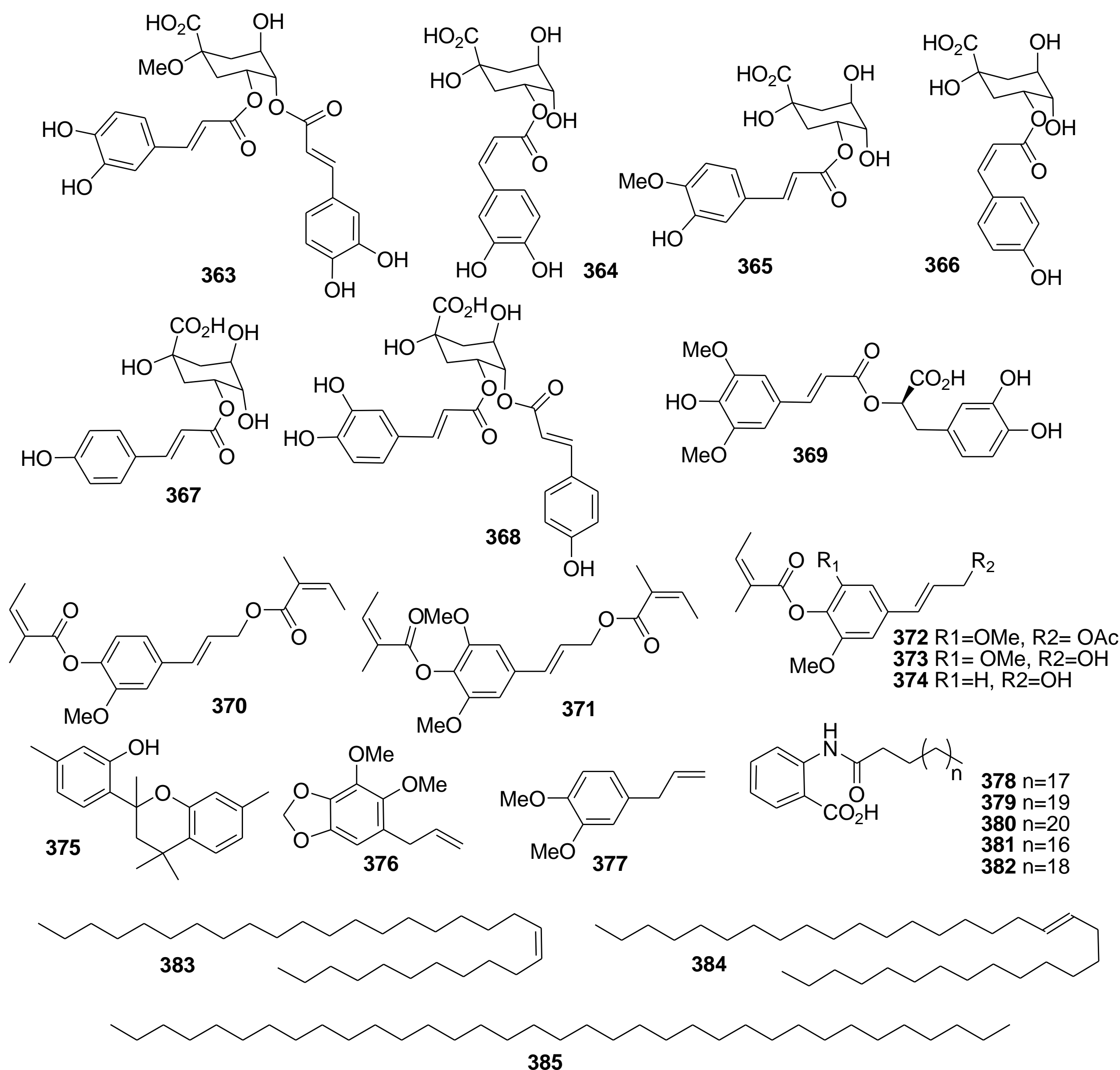


Fig. 12 Structures of miscellaneous compounds isolated from *Inula spp.* (cont)

Chemical yield of essential oils from *Inula spp.*

GC-MS analysis of the essential oil (0.5%) from *I. helenium* roots revealed that alantolactone (52.4%) (**20**) and isoalantolactone (33.0%) (**21**) were the main components in addition to minor compounds (3.1%) that include β-elemene (**386**), aromadendrene (**387**), β-selinene (**388**), elemol (**389**), caryophyllene oxide (**390**), myristic acid (**391**) and palmitic acid (**392**) [67]. Analogous analysis of leaf essential oil from *I. viscosa* identified major compounds as 12-carboxyeudesma-3, 11(13) diene (28.88%) (**393**), linolenic acid

(7.80%) (**394**), palmitic acid (5.38%) (**392**), butylhydroxytoluene (4.11%) (**395**) and fokienol (3.37%)(**396**) (Fig 13) [68].

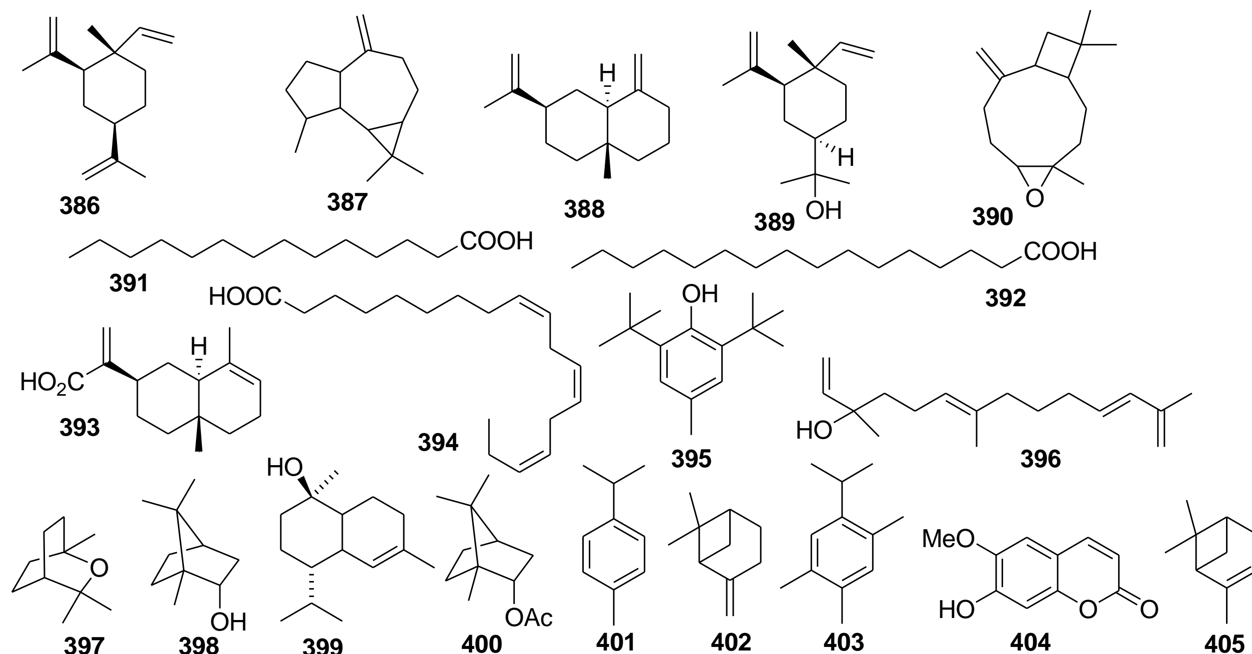


Fig 13. Structures of compounds present in the essential oil isolated from *Inula spp*.

The essential oil analysis of aerial parts of *I. graveolens* revealed the presence of 1, 8-cineole (**397**) (22.4%), borneol (**398**) (20.4%) and α-cadinol (**399**) (11.8%). Parallel study on *I. oculus-christi* yielded bornyl acetate (**400**)(21.3%), *p*-cymene (**401**)(16.6%) and β-pinene (**402**)(14.8%) as principal components. Similar investigations on *I. crithmoides* essential oil collected from central Italy indicated *p*-cymene (**401**) (30.1%), 1,2,4-trimethyl-5-isopropyl benzene (**403**) (18.7%), scopoletin (**404**) (15.3%) and α-pinene (**405**)(13.1%) [86'] as major components [56, 68]. GC-MS analysis of root essential oil of *I. britannica*, *I. salicina*, *I. bifrons*, *I. conyza* and *I. spiraeifolia* showed thymol (**233**) and its derivatives as major constituents [69].

1.5.2 Traditional uses and chemistry of the *Echinops spp*.

Roots of *E. Kebericho* (Asteraceae) are considered as one of the most important medicinal plants of Ethiopia and are among the commonly traded items in local markets. The characteristic smoke of the root is used to fumigate homes in order to get rid of insects and other disease causing pests [70]. The smoke is also used against typhus

and fever and to repel snakes from their vicinity. It is also indicated that the roots are chewed to reduce stomach ache in humans. A decoction of the roots is used to cure intestinal diseases in cattle. *In vitro* studies on other members of the genus showed strong antibacterial, nematicidal and molluscicidal activities [70]. It is also reported that plants in the genus showed termiticidal, antifungal, cytotoxic, antileishmanial activities and are also used for the treatment of hypertension [71-74].

Chemical investigations on *Echinops spp*. have resulted in the isolation of β-caryophyllene (**9**), dehydrocostus lactone (**108**), costunolide (**197**), 2,2':5',2''–terthiophene (**406**), acetylenic thiophenes (**11, 407-412**), echinothiophenegenol (**413**), coumarin (**414**), flavonoids (**327**, **415-419**), triterpenes (**291,307,308**,**420**) and alkaloids (**421**-**422**) (Table 4, Fig. 14) [71-73,75-78]. Previous chemical study on *E. kebericho* [79] demonstrated the presence of compounds **108** and **197**, of which the former is the major constituent. Thiophenes have been reported to possess antifungal and insecticidal activities [72, 80, 81].

Table 4. List of some isolated compounds from *Echinops spp*. (Root)

Compound class and name [cpd number]	**Source Echinops Species**	**Ref.**
β-caryophyllene([**9**)	*E. hispidus*	79
5'-(3-buten-1-ynyl)-2,2'-bithiophene (**11**)	*E. ritro, E. albicaulis, E. transiliensis, E. spinosissimus, E. macrochaetus*	71, 72, 79
Dehydrocostus lactone (**108**), costunolide [**197**]	*E. amplexicaulis, E. kebericho*	79
2,2':5',2''–terthiophene (**406**)	*E. ritro, E. albicaulis, E. transiliensis, E. macrochaetus*	71, 72
Isocardopatine (**407**)	*E. ritro, E. spinosissimus*	71, 72
4-[5-(penta-1,3-diynyl)thien-2-yl]-3-ynylbutanol (**408**)	*E. ritro, E. pappii*	71, 72
4-[5-(penta-1,3-diynyl)thien-2-yl]-2-chlorobut-3-ynyl acetate (**409**)	*E. transiliensis, E. hispidus*	71, 72
4-(2,2'-dithien-5'-yl)-2-acetoxybut-3-ynylacetate (**410**), 4-(2,2-dithien-5'-yl)-3-ynylbutanol (**411**), (2,2'-dithien-5'-yl)but-3-ynylisovalerate (**412**)	*E. ritro*	71, 72
Echinothiophenegenol (**413**)	*E. grijisii*	73
umbelliferone (**414**), Chrysoeriol (**415**), Hispidulin (**327**), Apigenin (**416**), Centaureidin (**417**), Jaceidin (**418**), Axillarin (**419**), Lupeol acetate (**420**), Lupeol (**291**), stigmasterol	*E. integrifolius.*	75

(**307**), β-sitosterol-3-glucoside (**308**), 7-hydroxyechinozolinone (**421**)		
1-Methyl-2,3-dihydro-4(1H)-quinolinone (**422**)	*E. heterophyllus*	78

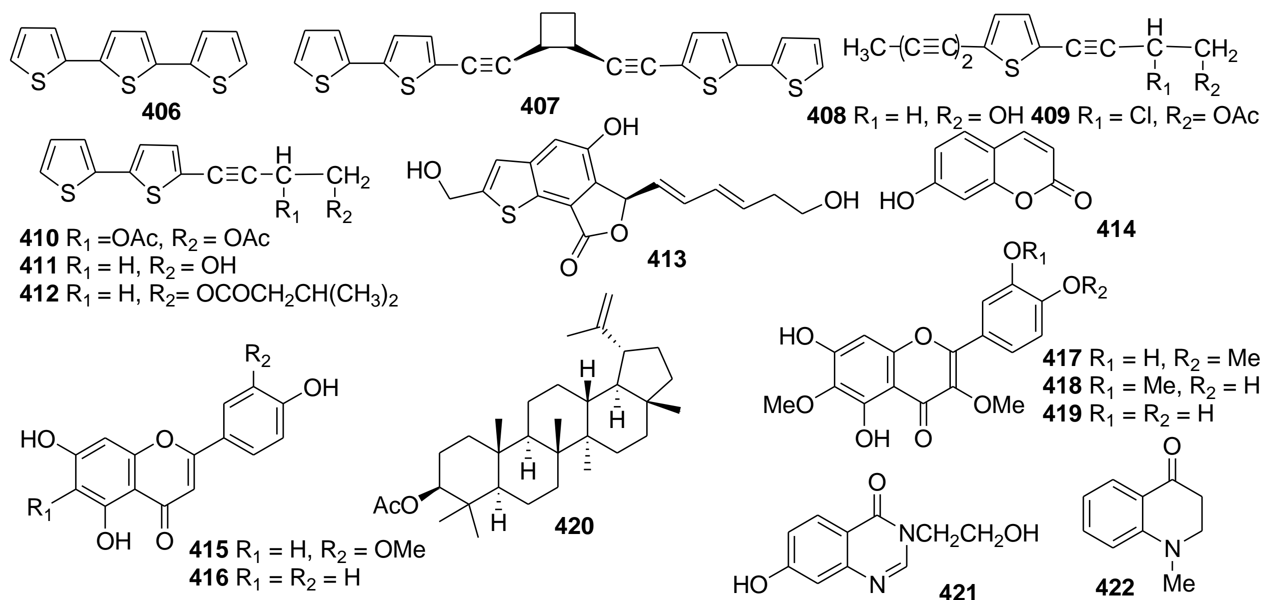


Fig. 14 Structures of compounds isolated from *Echinops spp*.

Structure-activity study on DHCL: *Saussurea lappa* (Asteraceae) is traditionally known for its medicinal uses in different indigenous Indian systems of medicine for its antiulcer, anti-convulsant, anti-cancer, hepatoprotective, anti-arthritic and anti-viral activities. It is popularly known as Kuth root or costus [82]. DHCL was isolated from the plant for the first time in 1964 and found to exhibit a variety of bioactivities including anticancer, antiviral, plant growth regulator and antibacterial activities [83]. Its first total synthesis was accomplished in 1984 [84].

Chemical and stereochemical modifications on DHCL showed marked effect on its activities. In the search for biologically active dehydrocostus lactone derivatives alkylation, double bond isomerization and epoxidation reaction products (Fig. 15) were examined. Alkylations of DHCL at conjugated C=C to lactone carbonyl using diazomethane produces pyrazolines (**423-424**) with better bioactivity as plant growth regulator [85, 86]. The literature reports correlate the enhanced activity with increased side chain length at C-13. For instance 13-Methyl derivative of DHCL (**425**) was found to be much more active than the parent molecule to promote root formation in the cuttings

of *Vigna radiate* [86]. In another study significant inhibitory activity of compound **108**, eremanthin (**426**) and isodehydrocostus lactone (**427**) on killing function of cytotoxic T lymphocytes was described. Molecules having *endo*-unsaturated γ-lactone (**428**) also showed lower activity as compared with their corresponding α-methylene-γ-lactones. Compounds with α-methyl-γ-lactones such as mokkolactone (**429**) exhibited no significant inhibitory activity on killing function of cytotoxic T lymphocytes [87]. In another antibacterial activity study, compound **108** showed higher inhibitory activity than its monoepoxides (**430-431**) and diepoxide (**432**) against *Mycobacterium tuberculosis* [88]**.** Isomerization reaction products (**433-434**), Michael addition adduct (**435**) and spirocyclopropyl analogues (**436-437**) were obtained and potential of Simmons-Smith reaction products was studied for the treatment of diabetes mellitus [85, 86, 89]**.** These observations assure the importance of α-methylene-γ-lactone moiety for the activity of the molecules, and it is an important structural subunit found in several sesquiterpene lactones with relevant biological activity due to its ability of alkylation with thiol groups found in proteins [90-93].

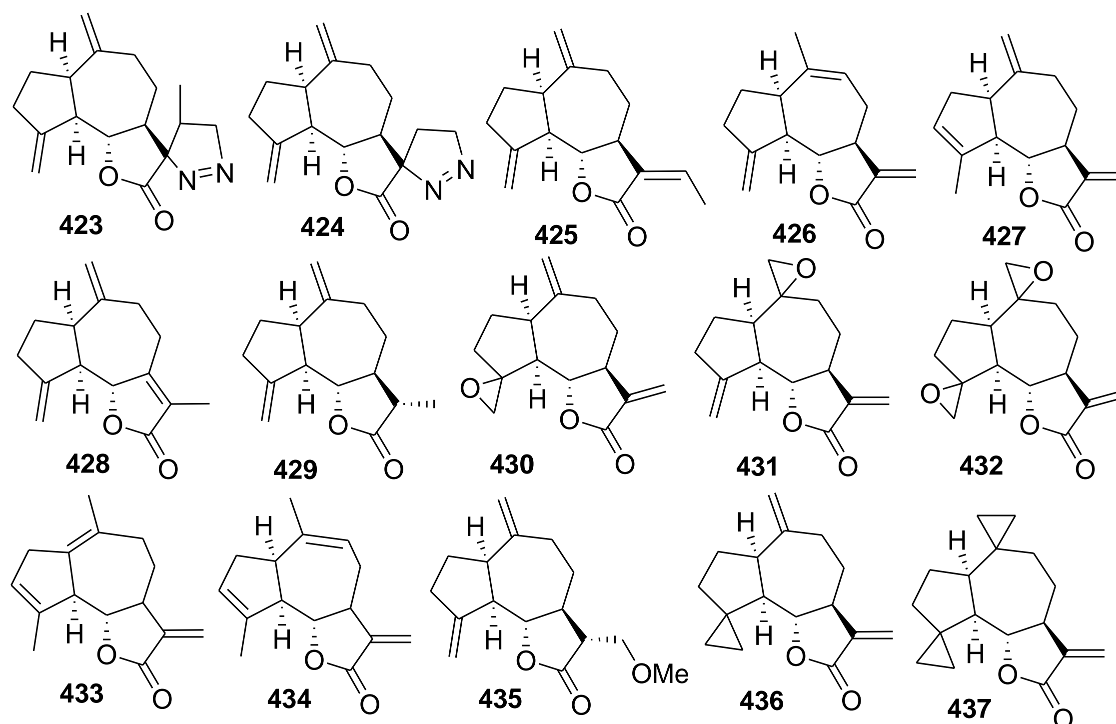


Fig. 15 Structures of derivatives of Dehydrocostus lactone

1.5.3 Review on Chemistry of *Artemisia annua* Leaf

Artemisia annua (Asteraceae) is originated in China, and now it is cultivated in many parts of the World including Ethiopia. In China *A. annua* is used for the treatment of fevers and as a source of medicine for treating malaria [94].

About 600 secondary metabolites were reported from *A. annua* in the literature comprising different structural groups. The investigations showed that the chemical composition of the plant is dominated by terpenoids (especially sesquiterpene lactones), coumarins and flavonoids which are largely responsible for the importance of this plant in medicine [95, 96].

A study on leaf essential oil of *A. annua* obtained from Bulgaria yielded mainly α-humulene (24.73%) (**438**), α-cuvebene (13.53%) (**439**), α-copaene (7.42%) (**440**), α-selinene (8.21%) (**441**), artemisia ketone (8.45%) (**442**) and camphor (3.61%) (**443**)[97] (Fig. 16). The Chinese variety reportedly contained predominantly **442** while the Vietnamese oil was dominated by **443** and germacrene D (**444**) [96-98].

Artemisinin (**445**), artemisitene (**446**), artemisinic acid (**447**), dihydroartemisinic acid (**448**), arteannuin B (**449**), dihydroarteannuin B (**450**), *Epi*-deoxyarteannuin B (**451**), dihydro-*Epi*-deoxyarteannuin B (**452**), dihydro-deoxyarteannuin B (**453**),deoxyarteannuin B (**454**), α-epoxyartemisinic acid (**455**) and α-epoxydihydroartemisinic acid (**456**) were isolated from *A. annua* of which artemisinin is biologically more active [96, 99, 100]. In addition to its potent antimalarial activity, it is also effective in treating other parasitic diseases, some viral infections and as allelopathic herbicide [100]. *Seco*-amorphane (**457**) and its 11,13-dihydro analogue (**458**), compounds **459-460**, deoxyartemisinin (**461**), arteannuin K (**462**), arteannuin L (**463**), arteannuin H (**464**), arteannuin I (**465**), annulide (**466**), arteannuin J (**467**), isoannulide (**468**), coumarin (**469**), scoparone (**470**), isofraxidin (**471**), tomentin (**472**), 6,7-Dimethoxydihydrocoumarin (**473**) were obtained in addition to polyacetylenes namely ponticaepoxide (**474**) and annuadiepoxide (**475**), scopolin (**381**), scopoletin (**404**), octacosanol and nonacosanol [96, 101] (Fig. 16).

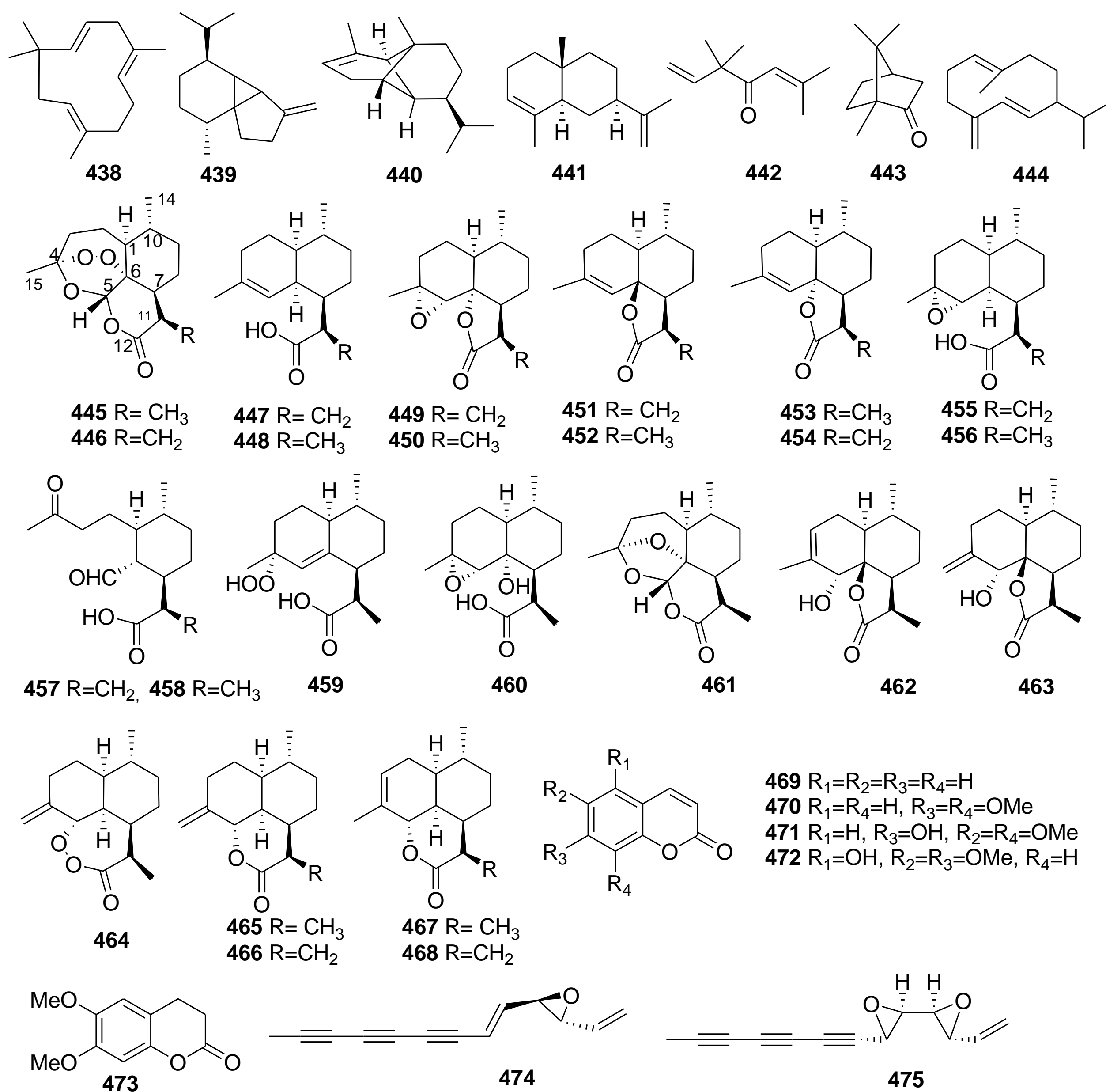


Fig 16. Structures of some compounds isolated from *Artemisia annua*

1.5.4 Essential oil composition of *Eucalyptus globulus* and *Thymus schimperi* Leaf

Eucalyptus: The genus Eucalyptus (Myrtaceae) comprises more than 700 species including *Eucalyptus globulus* (Nech Bahir Zaf (Amh)). In local medicine, the steam from boiled leaves is inhaled to relieve the common cold and other bronchial problems. Steam from *E. globulus* is believed to kill disease causing bacteria and microbes in sick rooms.

It is used as a steam inhalant for chest congestion [102]. Reportedly the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal [103]. The essential oil of the leaves of *E. globulus* yielded 1,8-cineole (eucalyptol) (>70%) (**397**) as the main ingredient together with *p*-cymene (**401**), α-pinene (**405**) and several other minor monoterpenes [104, 105].

Thymus: The genus Thymus (Lamiaceae) includes about 350 species worldwide. *Thymus vulgaris* is the common European garden plant whereas *T. schimperi and T. serrulatus* are endemic species of Ethiopia. *T. schimperi* (Tosign (Amh)/ Abyssinian Thyme (Eng)) occurs in the wild at high altitudes such as in Bale and Debre Sina [106]. The GC-MS analysis of the essential oil from the aerial parts of *T. schimperi* growing in Bale was found to contain thymol (50%) (**233**), γ-terpinene (12.1%), carvacrol (10.1%) and *p*-cymene (10.0%) as the major constituents whereas carvacrol (66.2%) and γ-terpinene (13.2%) were dominant in the same species obtained from Showa. Thymol (48.6%) and γ-terpinene (19.8%) were the major constituents of *T. vulgaris* oil grown in Wondo Genet [107]. GC-MS analysis of the essential oil obtained from *T. bornmuelleri* contained thymol (44.97%) as the main constituent [108-110].

1.6. Objectives of this study

The problems caused by synthetic fungicides have necessitated for alternative plant disease management strategies. In fact, plant secondary metabolites are potential sources of safer substitutes [3, 4, 13, 17, 18, 21, 25, 26]. Thus, investigations of different plant materials were of interest to find bioactive natural products against *F. spp* and *P. infestans* causing root rot/wilt and late blight, respectively. They are economically significant fungal plant diseases that treaten production of faba bean and potato in Ethiopia. There was no previous report in Ethiopia on phytochemical study for controlling plant diseases in general and root-rot/wilt and late blight in particular. Therefore, this study was intended to conduct bioassay guided isolation and characterization of compounds and essential oils responsible for the antifungal activities of the plant extracts against growth of *F. spp.* and *P. infestans* for use as sources of biofungicides in the control of root rot/wilt of faba bean and late blight of potato.

Taking these into considerations the specific objectives of the study were to:

- identify plant extracts/ essential oils with high antifungal activity on the basis of the preliminary *in vitro* bioassay study of plant crude extracts/ essential oils against the growth of *F. spp.* and *P. infestans*
- isolate and characterize compounds from chosen plant extracts
- determine antifungal activity of isolated compounds
- identify the part of the lead structure responsible for its antifungal activity by structure activity relation study of synthesized analogues
- isolate and identify major constituents of essential oils from plants studied using GC-MS analysis

2. Results and Discussion

A total of 57 plant species were selected for antifungal activity screening in this study. *In vitro* antifungal activity tests of crude extracts were undertaken using the optimized conditions and food poisoning bioassay method to find potential plants. The bioassay results of tested extracts against fungi causing root rot/wilt of faba bean and late blight of potato were analyzed. Accordingly, plants with higher fungal growth inhibitory potential were identified and some of them were subjected to bioactivity directed fractionations to isolate the active principles.

2.1 Optimization of the *in vitro* antifungal assay: Food poisoning method

The optimum working conditions of the bioassay method were established and appropriate solvent and synthetic fungicides were selected. In order to choose a better solvent system accessible solvents, namely, water, acetone, methanol, ethanol and dimethyl sulfoxide (DMSO) were tested at concentrations of 1, 5, 10, 20% (0.05, 0.25, 0,5,1.0 mL) in distilled water. In the course DMSO (5% in water) was found the better solvent to dissolve the materials tested and allowed better growth of the fungi (Growth inhibition, %GI ≤10). It was selected as negative control in all treatments and here after called vehicle (Fig. 17A and B). Synthetic fungicides available at APPRC (Mancolaxyl™, Ridom™, Unizeb™) were tested at doses of 0.1, 0.2, 0.5, 1.0% (1, 2, 5, 10 mg/mL in vehicle), and Mancolaxyl™ showed better inhibition potential (%GI=86-93 in *P. infestans* and 62-75% in *F. spp*) at about 0.5% concentration (5 mg /mL). It was selected as positive control here after called standard (Fig. 17C and D).

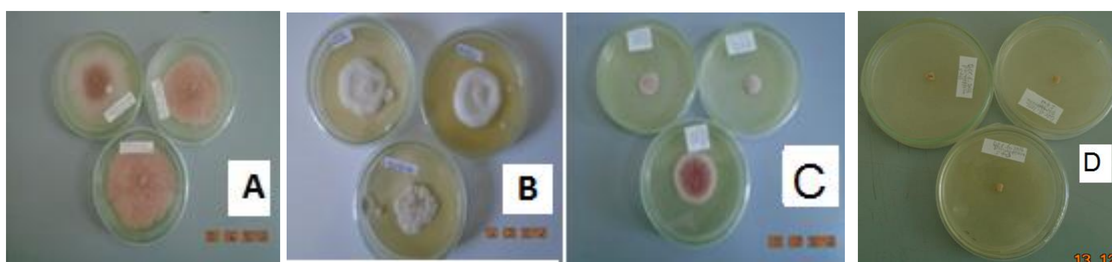


Fig. 17 Growth of *F. spp* (A,C) and *P. infestans* (B,D) in vehicle and standard

In this preliminary bioassay study, antifungal activities of different plant extracts were evaluated at concentrations of 1, 5, 10, and 20 mg/mL. No promising inhibition zone was

observed when extracts were applied at concentrations lower than 20 mg/mL. On the contrary the mycelial growth inhibitory activity was significant when the applied extract exceeded this amount. After testing several plants extracts the minimum concentration demonstrating measurable growth inhibitory activity (20 mg/mL) was selected as a minimum inhibition concentration (reference) for comparison of inhibition potential of extracts/ fractions. The amount of isolated compounds and essential oils tested was set equal to the amount of the standard (5 mg/mL) for comparison. The inoculum size (6 mm), incubation period (2 weeks) and temperature (26.4^{o}C) were adopted from APPRC mycology laboratory.

The maximum growth potential of each fungus was assessed using unamended growth medium (PDA) at optimum conditions (Fig. 18), and in the course the diameter of mycelial growth of *Fusarium spp.* was recorded in the range 64-90mm and that of *P. infestans* was 32-60 mm. The growth rate of *P. infestans* was relatively slow may be due to its need for selective growth media.

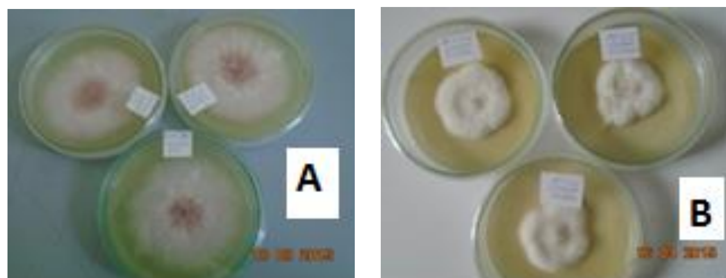


Fig. 18 Growth of *F. spp* (A) and *P. infestans* (B) in untreated media

2.2 Screening of plants against *Fusarium spp*. and *Phytophtra infestans*

2.2.1. Mycelial growth inhibition potential of plant extracts and essential oils

Ethanolic extracts of selected plants were subjected to *in vitro* antifungal test for their mycelial growth inhibition potential against *F. spp* using established condition. In this test towards the control of *F. spp.* plant extracts, seed oils and essential oils were included. The percentage of mycelial growth inhibition (%GI) of screened plant products is presented in Table 5.

Table 5. Mycelial growth inhibition ability of tested botanicals against *F. spp.*

S.No	Plant species name	Average mycelial growth (mm) and Mycelial growth inhibition percentage against *F.spp.*	
		G av±RSD	**%GI**
1	*Eucalyptus globulus* (EO)	16.0 ± 2.5	77.0
2	*Echinops kebericho*	19.0 ± 3.2	73.0
3	*Artemisia annua*	20.0 ± 2.0	71.0
4	*Premna schimperi*	21.0 ± 1.1	70.0
5	*Solanecio gigas*	21.0 ± 1.5	70.0
6	*Inula confertiflora* (Ap ext)	22.0 ± 1.5	69.0
7	*Thymus schimperi* (EO)	22.0 ± 1.0	69.0
8	*Olea europaea*	22.0 ± 1.1	69.0
9	*Syzygium guineense*	22.0 ± 4.5	69.0
10	*Jatropha curcas*	24.0 ± 1.5	66.0
11	*Parthenium hysterophorus*	27.0 ± 4.1	61.0
12	*Salvia schimperi* (Ap ext)	27.0 ± 3.1	61.0
13	*Cucumis ficifolius*	29.0 ± 1.5	58.0
14	*Eucalyptus globulus* (Lf ext)	30.0 ± 5.2	57.0
15	*A. indica* (Sd ext cake, oil)	30.0 ± 3.5	57.0
16	*Hagenia abyssinica*	30.0 ± 1.0	57.0
17	*Euclea divinorum*	30.0 ± 0.5	57.0
18	*Salvia schimperi* (Sd ext)	31.0 ± 2.6	56.0
19	*Azadirachta indica* (Lf ext)	31.0 ± 2.0	56.0
20	*Thymus schimperi* (Lf ext)	32.0 ± 2.5	52.0
21	*Inula confertiflora* (Rt ext)	34.0 ± 3.2	51.0
22	*Rumex nepalensis*	37.0 ± 9.2	47.0
23	*Melia azaderach*	39.0 ± 3.0	44.0
24	*Matricaria recutita*	41.0 ± 1.5	42.0
25	*Rhamnus prinoides*	43.0 ± 3.7	38.0
26	*Maesa lanceolata*	45.0 ± 3.0	35.0
27	*Balanites aegyptiaca*	48.0 ± 1.1	32.0
28	*Lepidium sativum*	48.0 ± 1.1	32.0
29	*Leonotis ocymifolia*	48.0 ± 4.0	32.0
30	*Salvia officinale*	50.0 ± 4.3	29.0
31	*Cynoglossum coeruleum*	50.0 ± 2.5	29.0
32	*Warburgia ugandensis*	50.0 ± 1.0	28.0
33	*Tagetes minuta*	51.0 ± 4.5	27.0
34	*Guizotia scabra*	51.0 ± 4.0	27.0
35	*Galium simense*	51.0 ± 1.5	27.0
36	*Chenopodium ambrosodes*	52.0 ± 1.6	25.0
37	*Galiniera saxifrage*	53.0 ± 5.0	24.0
38	*Rumex abyssinicus*	54.0 ± 3.0	23.0
39	*Bersama abyssinica*	55.0 ± 1.5	22.0
40	*Kniphofia foliosa*	55.0 ± 2.0	21.0
41	*Rumex nervosus*	55.0 ± 2.5	21.0
42	*Bidens macroptera*	55.0 ± 5.5	21.0
43	*Ricinus communis*	57.0 ± 3.2	19.0
44	*Cordia Africana*	58.0 ± 2.0	18.0
45	*Achyranthes aspera*	58.0 ± 4.0	17.0
46	*Vernonia amygdalina*	59.0 ± 1.4	16.0
47	*Olinia rochetiana*	59.0 ± 1.5	15.0
48	*Myrsine Africana*	60.0 ± 5.5	14.0
49	*Millettia ferruginea*	61.0 ± 5.5	13.0
50	*Zehneria scabra*	62.0 ± 3.0	12.0
51	*Osyris quadripartite*	62.0 ± 2.6	12.0

52	*Withania somnifera*	62.0 ± 2.0	11.0
53	*Datura stramonium*	63.0 ± 2.0	10.0
54	*Lantana camara*	64.0 ± 5.1	09.0
55	*Uritica dioca*	64.0 ± 4.1	09.0
56	*Croton macrostachyus*	64.0 ± 2.6	09.0
57	*Allophylus abyssinicus*	64.0 ± 2.0	09.0
58	*Verbascum sinaiticum* (Fr, Lf)	65.0 ± 0.5	07.0
59	*Moringa stenopetala*	65.0 ± 0.5	07.0
60	*Plantago lanceolata*	66.0 ± 4.7	05.0
61	*Sesbania sesban*	66.0 ± 5.5	05.0
62	*Thalictrum rhynchocarpum*	66.0 ± 0.8	05.0
63	Control (DMSO)	70.0 ± 3.0	10.0
64	Mancolaxyl® (std)	22.0 ± 2.6	68.0
65	PDA	70.0 ± 3.6	-

Rsults are mean ± Relative standard deviation (%RSD), each experiments performed in triplicate

Plant extracts showed varied degrees of mycelial growth inhibition. Of the tested plant extracts *Millettia ferruginea* (13%), *Zehneria scabra* (12%), *Osyris quadripartite* (12%), *Withania somnifera* (11%), *Datura stramonium* (10%), *Lantana camara* (9%), *Croton macrostachyus* (9%), *Uritica dioca* (9%), *Allophylus abyssinicus* (9%), *Plantago lanceolata* (5%), *Sesbania sesban* (5%), *Thalictrum rhynchocarpum* (5%), *Verbascum sinaiticum* (7%) and seed oil from *Moringa stenopetala* (7%) showed lowest antifungal activity (%GI<14%) relative to the control and categorized as inactive (Fig 19). In the test, 27 plants showed %GI in the range of 14-50% and they were categorized as weak (Fig 19). These are *Rumex nepalensis* (47%), *Melia azaderach* (44%), *Matricaria recutita* (42%), *Rhamnus prinoides* (38%), *Maesa lanceolata* (35%), *Balanites aegyptiaca* (32%), *Lepidium sativum* (32%), *Leonotis ocymifolia* (32%), *Salvia officinale* (29%), *Cynoglossum coeruleum* (29%), *Warburgia ugandensis* (28%), *Tagetes minuta* (27%), *Guizotia scabra* (27%), *Galium simense* (27%), *Chenopodium ambrosoides* (25%), *Galiniera saxifrage* (24%), *Rumex abyssinicus* (23%), *Bersama abyssinica* (22%), *Kniphofia foliosa* (21%), *Rumex nervosus* (21%), *Bidens macroptera* (21%), *Ricinus communis* (19%), *Cordia Africana* (18%), *Achyranthes aspera* (17%), *Vernonia amygdalina* (16%), *Olinia rochetiana* (15%) and *Myrsine Africana*) (14%).

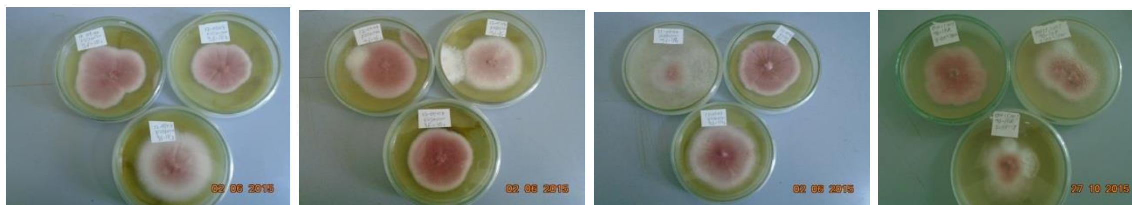


Fig 19. *In vitro* growth inhibition potential of *Lantana camara, Croton macrostachyus, Plantago lanceolata* and *Lepidium sativum,* respectively

Leaf extracts of *Thymus schimperi* (52%), *Azadirachta indica* (56%), *Eucalyptus globulus* (57%), *Euclea divinorum* (57%), *Parthenium hysterophorus* (61%), *Salvia schimperi* (61%), and extracts of *Inula confertiflora* (RT) (51%), *Cucumis ficifolius* (FR) (58%), *Hagenia abyssinica* (FL) (57%) showed %GI of 50-68 and they were considered as moderately active compared with the control. Similarly seed oils of *Jatropha curcas* (66%) and *Azadirachta indica* (57%), and seed extracts of *Azadirachta indica* (57%) and *Salvia schimperi* (56%) were moderately active (Fig. 20). All the *Azadirachta indica* samples showed closer antifungal activity.

Fig. 20. *In vitro* growth inhibition potential of extracts of *T. schimperi* (LF), *A. indica* (SD cake), *J. curcas* (SD oil) and *E. globulus* (LF), respectively

Extracts of *Echinops kebericho* (RT) (73%), *Artemisia annua* (LF) (71%), *Premna schimperi* (LF) (70%), *Solanecio gigas* (LF) (70%), *Inula confertiflora* (AP) (69%), *Syzygium guineense* (LF) (69%) and *Olea europaea* (LF) (69%) demonstrated similar inhibitory activity with the standard fungicide (Fig. 21).

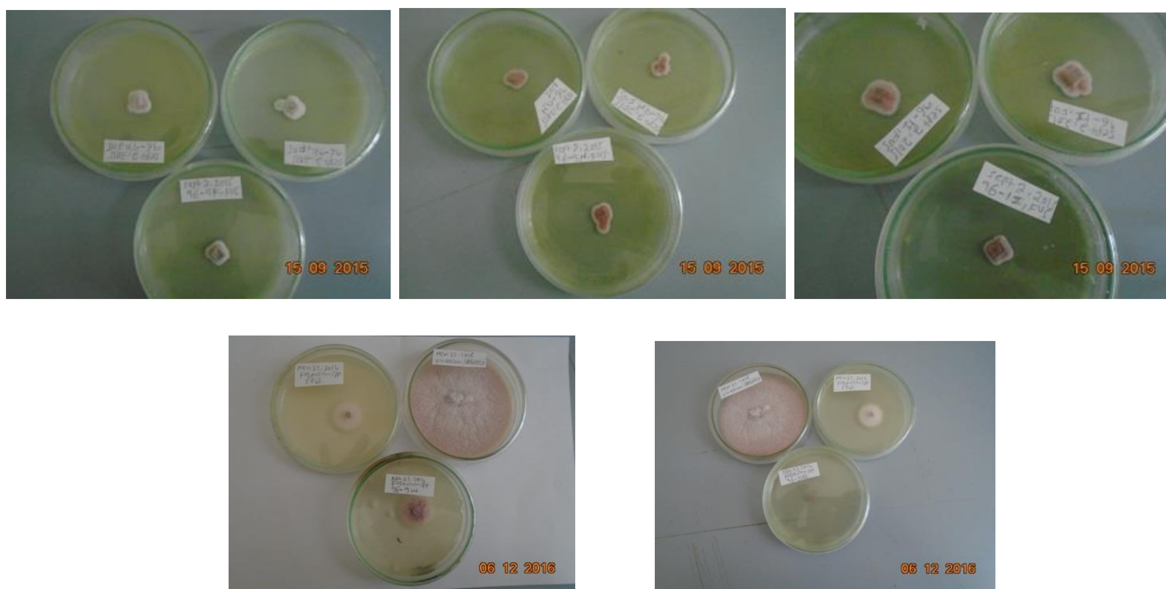


Fig. 21 *In vitro* growth inhibition potential of *E. kebericho*, *A. annua*, and *I. confertiflora*, *E. globulus* (EO) and *T. schimperi* (EO), respectively.

Percentage of growth inhibition was also recorded for leaf essential oils (EO) from *Eucalyptus globulus* (77%) and *Thymus schimperi*) (69%). They were found comparable to the standard fungicide in the control of *F. spp.*at a concentration of 5 mg/mL (Fig. 21). The plant materials with growth inhibitory activity of 69% and above were considered as highly active plants against *F. spp,* and they were subjected to futher investigations.

Similarly, the following plant materials were assayed against mycelial growth of *P. infestans* at optimum conditions and their %GI is tabulated in Table 6.

Table 6. Mycelial growth inhibition ability of tested botanicals against *P. infestans*

S.no	**Ext code**	**Average mycelial growth (mm) and mycelial growth inhibition percentage against *P. infestans***	
		Gav± RSD	**%GI**
1	*Eucalyptus globulus* (EO)	05.0 ± 0.5	88.0
2	*Thymus schimperi* (EO)	06.0 ± 1.5	86.0
3	*Echinops kebericho*	09.0 ± 1.5	80.0
4	*Inula confertiflora* (Ap ext)	10.0 ± 1.0	78.0
5	*Thymus schimperi* (Lf ext)	11.0 ± 2.8	75.0
6	*Artemisia annua*	12.0 ± 3.5	73.0
7	*Hagenia abyssinica*	20.0 ± 1.5	55.0
8	*Eucalyptus globulus* (Lf ext)	22.0 ± 1.7	52.0
9	*Croton macrostachyus*	25.0 ± 1.7	44.0
10	*Lantana camara*	27.0 ± 1.0	39.0
11	*Parthenium hysterophorus*	31.0 ± 4.5	31.0
12	*Azadirachta indica*	32.0 ± 8.5	29.0
13	*Leonotis ocymifolia*	33.0 ± 3.5	26.0
14	*Rumex nepalensis*	34.0 ± 2.8	25.0
15	*Salvia schimperi*	35.0 ± 4.0	21.0
16	*Plantago lanceolata*	36.0 ± 3.6	20.0
17	*Olea europaea*	36.0 ± 3.8	19.0
18	*Melia azaderach*	39.0 ± 3.7	13.0
19	*Galiniera saxifrage*	40.0 ± 7.6	18.0
20	Control (DMSO)	45.0 ± 1.4	02.0
21	Mancolaxyl®	05.0 ± 1.0	88.0
22	PDA	46.0 ± 1.2	-

Rsults are mean ± Relative standard deviation (%RSD), each experiments performed in triplicate

In this *in vitro* bioassay test, leaf extracts of *Croton macrostachyus* (44%)*, Lantana camara*) (39%), *Parthenium hysterophorus* (31%), *Azadirachta indica* (29%), *Leonotis ocymifolia* (26%) (Fig 22), *Rumex nepalensis* (25%), *Salvia schimperi* (21%)*, Plantago lanceolata* (20%), *Olea europaea* (19%), *Galiniera saxifrage* (18%) and *Melia azaderach* (13%) demonstrated %GI < 50 and categorized as inactive. In the same way extracts of *Hagenia abyssinica* (FL) (55%) and *Eucalyptus globulus* (LF) (52%) were subjected to antifungal assay and showed moderate activity against *P. infestans*.

Fig. 22 Inhibition potential of *L.ocymifolia* (AP) and *A. indica* (LF)

Leaf extracts of *Artemisia annua* (73%), *Thymus schimperi* (75%), *Inula confertiflora* (78%) and root extract of *Echinops kebericho* (80%) displayed higher antifungal activities against the growth of *P. infestans* as shown in Table 6.

Leaf essential oils from *E. globulus* and *T. schimperi* also showed similar mycelial growth inhibitory activity (%GI > 85) as the standard fungicide ((%GI = 88) against late blight causing fungi (*P. infestans*). Fig. 23 illustrates the antifungal activities of leaf extracts of *E. kebericho* and *I. confertiflora,* and essential oils from *E. globulus* and *T.schimperi.* These better-acting plants extracts/ essential oils were selected for chemical study to identify the compound (s) responsible for the observed antifungal activity.

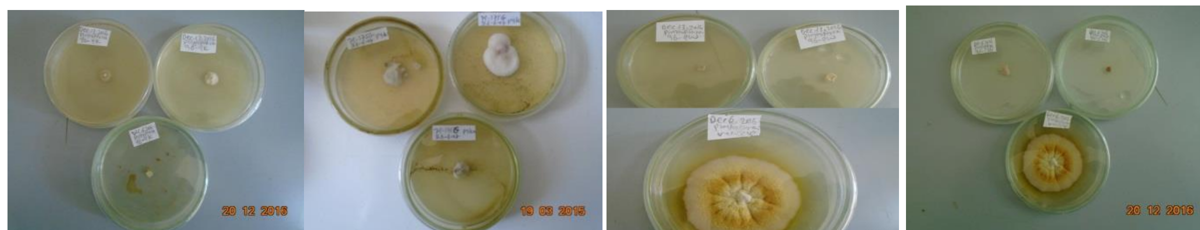


Fig. 23 Inhibition potential of *E. kebericho*, *I. confertiflora*, *E. globulus* and *T.schimperi*

2.2.2. Fractionation of selected plant extracts for bioassay guided chemical study

Based on the *in vitro* antifungal activity test conducted at APPRC, extracts of *Inula confertiflora, Echinops kebericho*, *Artemisia annua*, and essential oils of *Eucalyptus globulus* and *Thymus schimperi* were found active against both fungi. As a result the first three were selected and fractionated as follows for further bioassay-directed chemical study. Ethanol extracts of *I. confertiflora, E. kebericho* and *A. annua* were fractionated into nonpolar and polar portions using hexane/chloroform (1:1) and methanol, respectively and subjected to antifungal activity test against test fungi by the optimized bioassay method. Similarily, ethanol crude extracts and essential oils from *E. globulus* and *T. schimperi* were also analyzed. The result obtained from antifungal assay against growth of *F. spp* and *P. infestans* is shown in Table 7.

Table 7. Growth inhibition activity (%GI) of extracts and fractions (EOs) of selected plants

S.No	Crude extract and solvent fractions	Average mycelial growth (mm) and growth inhibition percentage (*Fusarium spp.*)		Average mycelial growth (mm) and growth inhibition percentage (*P. infestans*)	
		G_{av} ± RSD	%GI	G_{av} ± RSD	%GI
1	***I. confertiflora* (LF)**	22.0 ± 1.5	69.0	10.0 ± 1.0	78.0
	Hex + $CHCl_3$ (1:1)	18.0 ± 1.0	75.0	13.0 ± 3.7	71.0
	MeOH	46.0 ± 5.5	34.0	29.0 ± 0.5	35.0
2	***E. kebericho* (RT)**	19.0 ± 3.2	73.0	09.0 ± 1.5	80.0
	Hex + $CHCl_3$ (1:1)	17.0 ± 1.5	76.0	11.0 ± 1.0	76.0
	MeOH	44.0 ± 4.1	37.0	24.0 ± 1.0	46.0
3	***A. annua* (LF)**	20.0 ± 2.0	71.0	12.0 ± 3.5	73.0
	Hex + $CHCl_3$ (1:1)	22.0 ± 3.7	69.0	10.0 ± 2.5	78.0
	MeOH	34.0 ± 9.0	51.0	27.0 ± 3.6	40.0
4	***E. globulus* (LF)**	30.0 ± 5.2	57.0	22.0 ± 1.7	52.0
	(EO)	16.0 ± 2.5	77.0	05.0 ± 0.5	88.0
5	***T. schimperi* (LF)**	32.0 ± 0.8	52.0	11.0 ± 2.8	75.0
	(EO)	22.0 ± 1.0	69.0	06.0 ± 1.5	86.0
6	**Control (DMSO)**	70.0 ± 3.0	10.0	45.0 ± 1.4	02.0
7	**Mancolaxyl® (std)**	22.0 ± 2.6	68.0	05.0 ± 1.0	88.0

Rsults are mean ± Relative standard deviation (%RSD), each experiments performed in triplicate

According to the bioassay test (Table 7), nonpolar fractions of *I. confertiflora, E. kebericho* and *A. annua* were found more potent (%GI > 68) than their polar counterpart (%GI < 52). Similarly, leaf essential oils from *E. globulus* and *T. schimperi* were also promising in their *in vitro* fungal growth inhibititory activity. As result nonpolar fractions of the above listed plant extracts were chosen for phytochemical study to isolate the active principles. In addition the aforementioned active essential oils were also subjected to GC-MS analysis to identify their major chemical components.

2.3 Characterization of compounds and essential oils

2.3.1 Characterization of compounds isolated from *Inula confertiflora*

Inula confertiflora (Asteraceae, Fig. 24) locally named as *Weynagift* (*Amh*) was collected from Ankober Palace Lodge located in Ankober, North Shewa, Ethiopia. It was identified by professional botanist and voucher specimen (Voucher No. S1152) was deposited at the National Herbarium of Ethiopia, AAU. Other than the antifungal activity observed in this work and antiviral activity study elsewhere [58], no prior chemical study reports were found on this endemic plant.

Fig. 24 *Inula confertiflora* (aerial, root, flower) (Photo by MG)

Phytochemical investigations on the nonpolar fraction of the leaf crude extract afforded six compounds, namely, *n*-hentriacontane (**476**), β-sitosterol (**306**), stigmasterol (**307**), graveolide (**131**), carabrone (**174**) and carpesiolin (**139**). Sesquiterpene lactones **131, 139, 174** and phytosterol **307** were the major metabolites found in all parts of *Inula confertiflora*. Similar chemical study on the root extract revealed the presence of the major compounds mentioned above, thymol (**233**), epifriedelanol (**241**) and dammara-20,24-dien-3-yl acetate (**245**). In addition to the ubiquitous compounds of the species inuviscolide (**107**) and 4, 5-Epoxyinuviscolide (**122**) were also isolated from the flower extracts. The structures of these compounds were elucidated by using spectroscopic data and through comparison with data reported in the literature as follows.

Compound 476

Compound **476** was obtained as white waxy solid (Rf 0.81 in hex:EtOAc (1:1); mp 60-63°C) from the nonpolar fraction of the ethanol extract of leaves of *I. confertiflora*. The EI-MS of **476** revealed a molecular ion peak at m/z 436 corresponding to the elemental composition $C_{31}H_{64}$. Base peak was observed at m/z 57 (100%, $C_4H_9^+$). Another prominent fragment ion was observed at m/z 43 (65%, $C_3H_7^+$). Moreover, a fragmentation pattern showing a difference of 28 mass units corresponding to the loss of ethylene was observed which is typical of long chain hydrocarbons. The UV spectrum of the compound indicated the absence of UV-active chromophores. The IR spectrum showed only bands for C-H stretching vibrations (2923/2849 cm^{-1}). The spectrum showed no absorption bands for carbonyl, carboxylic acid, alcohol, amine and multiple bonds. The ^1H NMR spectrum ($CDCl_3$) of the compound displayed a triplet signal at δ0.90 integrated for two methyl protons adjacent to methylene carbon and a signal at δ1.27 (br s) showed overlapped long chain methylene protons (58H).

The ^{13}C and DEPT-135 NMR ($CDCl_3$) revealed the presence of five carbon signals. The signal at δ14.1 was assigned to terminal methyl carbon and the other four signals at δ22.7, 29.3, 29.7 and 31.9 were identified as methylene carbons. The more intense signal at δ29.7 represented the existence of many overlapped methylene carbons. The above data suggested that the compound isolated was most likely a long chain alkane and the carbon signals were ascribed to CH_3 (C-1, 31), CH_2 (C-2, 30), CH_2 (C-4, 28), CH_2(C-5-27) and CH_2(C-3, 29). The overall data was found similar with literature values of *n*-hentriacontane (**476**) (Table 8). Its isolation from *Inula spp*. was not reported in the literature.

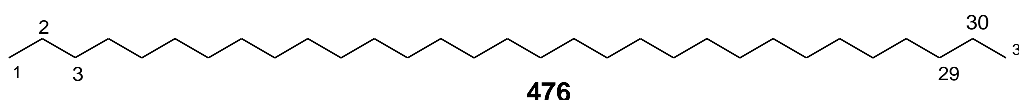


Table 8.The ^{1}H- and ^{13}C-NMR data of compound **476** and the data reported in the literature for n-hentriacontane ($CDCl_3$, δ in ppm, J in Hz) [111, 112]

Carbon no	NMR data of 476 δH	δC	NMR data of n-hentriacontane δH	δC
1,31	0.90 (t, $2CH_3$, J=6.4)	14.1	0.88	14.1
2,30	1.27 (br s,$2CH_2$)	22.7	1.25	22.7
3,29	1.27 (br s,$2CH_2$)	31.9	1.25	31.9
4,28	1.27 (br s,$2CH_2$)	29.3	1.25	29.3
5-27	1.27 (br s,$23CH_2$)	29.7	1.25	29.7

Compound 306

Compound **306** was obtained as white solid after recrystalization from MeOH/$CHCl_3$ (Rf 0.48 in hex: EtOAc (1:1); mp 134-135°C). The IR spectrum indicated the presence of OH (3430 cm^{-1}), C=C (1693 cm^{-1}) and C-H stretching vibrations (2930/2843 cm^{-1}). The ^{1}H-NMR spectrum ($CDCl_3$) of the compound showed six methyl proton signals at δ 1.03 (3H, s), δ 0.70 (3H, s), δ 0.94 (3H, *d*, J=6.4 Hz), and δ 0.85 (9H, m) which are assignable for H-19, H-18, H-21, H-26, H-27, H-29, respectively. The multiplets at δ 3.53 and 5.37 displayed protons at oxygenated carbon (H-3) and a double bond (H-6), respectively. The ^{13}C NMR spectrum ($CDCl_3$) showed twenty seven resolved carbon signals. The DEPT-135 spectrum revealed that these signals are due to six methyl, nine methine, ten methylene and two quaternary carbons (Table 9). The spectrum has recognizable signals at δ 140.7 and 121.7 which were assigned to double bonds at C-5 and C-6, respectively. The NMR data indicated the presence of sterol nucleus with one double

bond and the data was found consistent with literature values (Table 9) for β-sitosterol (**306**). It is widely distributed in plants and is the commonest sterol of higher plants. It is demonstrated to show antihypercholesterolaemic, estrogenic and hypolipidaemic agent besides its antimicrobial activity (DNP).

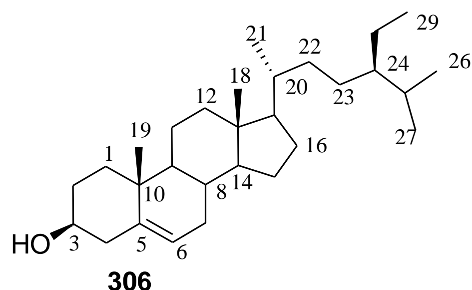


Table 9. The ^{13}C-NMR data of compound **306** and the data reported in the literature for β-sitosterol ($CDCl_3$, δ in ppm) [65]

C no	NMR data of 306 ^{13}C-NMR	NMR data of β-sitosterol ^{13}C-NMR	C no	NMR data of 306 ^{13}C-NMR	NMR data of β-sitosterol ^{13}C-NMR
1	37.2	37.3	**16**	28.2	28.2
2	31.7	31.7	**17**	56.1	56.1
3	71.8	71.6	**18**	11.9	11.9
4	42.3	42.3	**19**	19.4	19.4
5	140.7	140.7	**20**	36.1	36.1
6	121.7	121.7	**21**	19.0	19.1
7	31.7	31.7	**22**	33.9	34.0
8	31.9	31.9	**23**	26.1	26.2
9	50.1	50.2	**24**	45.8	45.9
10	36.5	36.5	**25**	29.1	29.2
11	21.1	21.1	**26**	18.8	18.8
12	39.8	39.8	**27**	19.8	19.8
13	42.3	42.3	**28**	23.1	23.1
14	56.8	56.8	**29**	12.0	12.0
15	24.3	24.3			

Compound 307

Compound **307** was found as colorless solid after recrystalization from EtOAc (Rf 0.48 in hex: EtOAc (1:1); mp145-147°C). In its IR spectrum, very intensely sharp bands at 3456 $cm^{-1}$and 1619 cm^{-1} were observed for the O-H and C=C vibrations, respectively. The C-H bond vibrations were also noticed by the bands at 2956/2856 cm^{-1}. In its ^{1}H-NMR spectrum ($CDCl_3$) a proton attached to oxygenated carbon (H-3) appeared as a doublet of doublet of doublets at δ 3.55 (*J*=16.0,11.2,4.8 Hz) and an olefinic proton (H-6) was also observed as multiplet at δ 5.36. Two additional olefinic proton signals were also seen at δ 5.03 (*dd*, *J*=15.2, 8.4Hz) and 5.17 (*dd*, *J*= 15.2, 8.4 Hz) and represented

coupled protons of C-22 and C-23 of the sterol nucleus. The spectrum also showed six methyl proton signals at δ 0.71 (3H, s), 0.82 (9H, m), 1.00 (3H,s), 1.03 (3H,m) and were assignable to H-18, H-21, H-27, H-29, H-19 and H-26, respectively. The ^{13}C-NMR spectrum ($CDCl_3$) showed twenty seven carbon signals. Two carbon signals were assumed to be overlapped at two intense signals located at δ31.9 and 21.1. The DEPT-135 NMR spectrum exhibited six methyl, ten methine, nine methylene and three quaternary carbon signals. It also showed signals at δ 140.7 and 121.7 which are assigned to C-5 and C-6 double bonds, respectively. The signals which appeared at δ 138.3 and 129.3 showed the presence of additional olefic carbons assignable to C-22 and C-23. Absence of olefinic carbon resonance around δ117.0 confirmed that compound **307** could possibily stigmasterol. Table 10 compares ^{1}H and ^{13}C NMR data of **307** with those reported in the literature [113] for stigmasterol and a reasonably good agreement was observed.

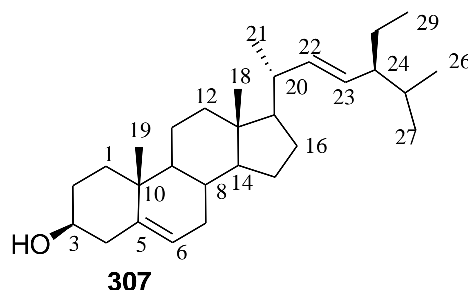


Table 10. The ^{13}C-NMR data of compound **307** and the data reported in the literature for stigmasterol ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz) [113]

C no	NMR data of 307 ^{13}C-NMR	NMR data of stigmasterol ^{13}C-NMR	C no	NMR data of 307 ^{13}C-NMR	NMR data of stigmasterol ^{13}C-NMR
1	37.3	37.6	**16**	28.9	28.7
2	31.7	32.0	**17**	56.0	56.3
3	71.8	72.2	**18**	12.3	12.3
4	42.3	42.7	**19**	19.4	19.4
5	140.8	141.1	**20**	40.5	41.0
6	122.0	122.1	**21**	21.1	21.5
7	29.7	32.0	**22**	138.3	138.7
8	31.9	32.3	**23**	129.3	129.6
9	50.2	50.5	**24**	51.2	51.6
10	36.5	36.6	**25**	31.9	32.3
11	21.1	21.5	**26**	21.2	21.5
12	39.7	40.0	**27**	19.0	19.2
13	42.2	42.6	**28**	25.4	25.8
14	56.9	57.2	**29**	12.1	12.4
15	24.4	24.7			

Compound 131

Compound **131** was isolated as white crystals (Rf 0.39 in EtOAc:Hex (1:1); mp 142-145°C). The IR spectrum showed absorption bands at 1769 cm^{-1} (γ-lactone), 1720 cm^{-1} (ring ketone), 1656 cm^{-1} (C=C) and 2981/2930 cm^{-1} (C-H) indicating the presence of α, β-unsaturated γ-lactone function in the compound. The ^{1}HNMR spectrum showed signals integrating for twenty hydrogens. In the spectrum two methyl proton signals at δ1.04 (3H,*s*) and 1.08 (3H,*d, J* = 6 Hz) were observed. The spectrum also exhibited two characteristic doublets (Fig. 25) at δ 6.18 and 5.51 (*J*= 3.6 and 3.2 Hz) corresponding to protons of exocyclic methylene group conjugated with a γ-lactone.

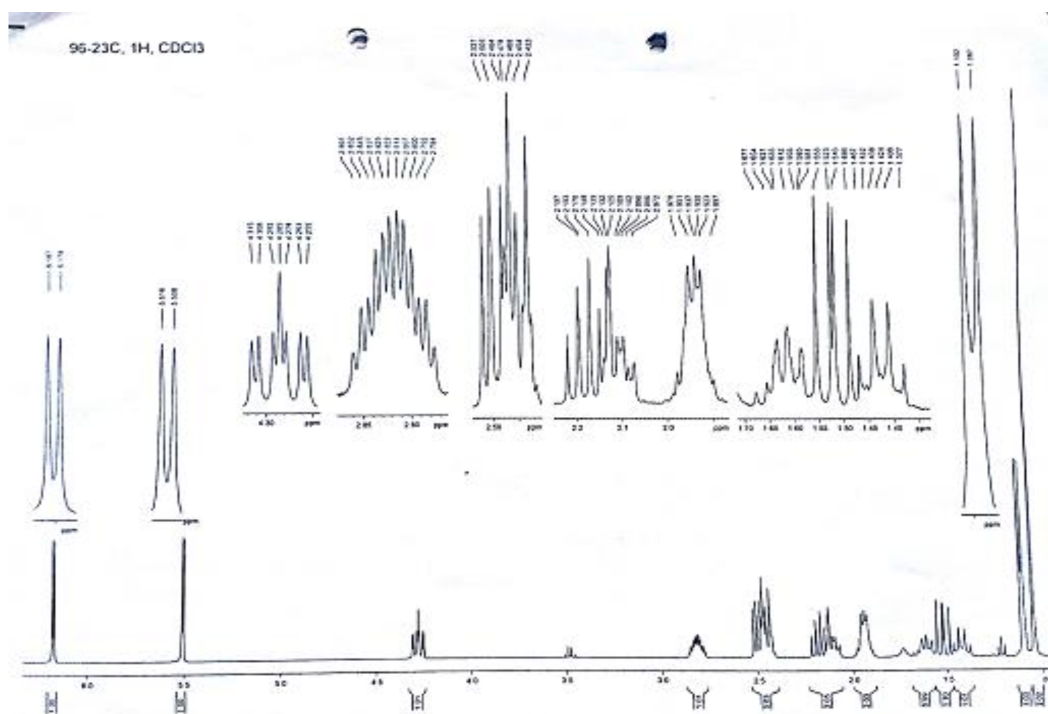


Fig 25. Expanded ^{1}H NMR spectrum of compound **131**

Proton signals at δ2.81 (*qt, J*= 12.4, 9.6, 3.6, 3.2Hz) and 4.28 (*ddd, J*=12.0, 9.2, 3.2Hz) were also observed in the spectrum ascribable to H-7 and H-8, respectively. The ^{13}C NMR spectrum (CDCl$_3$) of the compound showed a total of 15 carbon signals and the DEPT-135 experiment identified them as two methyls (δ 20.0, 22.0), four methines (δ 29.6, 44.7, 48.7, 80.8), five methylenes (δ 24.1, 34.5, 35.2, 44.1, 120.1) and four quaternary carbons (δ 50.0,140.2,169.9 and 221.2). The signals at δ80.8 and 221.2

confirmed the presence of oxymethine and ring ketone, respectively. The presence of a lactone function in the compound was readily recognized from the ^{13}C NMR chemical shift at δ169.9 (C-12) of the lactone carbonyl group. The signals at δ120.1 and 140.2 were ascribable to a terminal double bond. Thus, the existence of α-methylene-γ-lactone and cyclic ketone functions were evident from the data. The LC-ESI mass spectrum exhibited $[M+H]^+$ peak at m/z 249.14. This, in combination with ^{1}H and ^{13}C NMR data, indicated a molecular formula of $C_{15}H_{20}O_3$ with six degrees of unsaturation. Three of the unsaturations were due to three double bonds and the remaining three must be due to three rings.

Based on the above data, a sesquiterpene lactone of pseudoguaianolide skeleton was anticipated and this structure was well supported by 2D NMR analyses including ^{1}H-^{1}H COSY and HMBC experiments. In the ^{1}H-^{1}H COSY spectrum of the compound correlations were observed between methyl proton signal at δ1.08 (3H,*d*, $J = 6$Hz, H-14) and methine multiplet at δ1.94 (1H, H-10). Correlations of methine signal at δ 2.81 (H-7) with signals at δ2.17 and 2.48 (H-6) and δ4.28 (H-8) were clearly shown. The allyllic couplings between H-7 and δ6.18 and 5.51 (H-13) indicated the position of the exocyclic methylene group. The correlations of a multiplet signal at δ1.63 (H-2) with signals at δ1.94 (H-1), δ2.10 (H-2) and δ2.48 (H-3), and couplings of H-8 with H7, δ1.42 and 2.48 (H-9) were also observed in ^{1}H-^{1}H COSY spectrum. The exocyclic double bond proton signals (H-13) showed HMBC correlations with the carbonyl carbon resonated at δ169.9 (C-12) and quaternary olefinic carbon at δ140.2 (C-11) which was evident for the presence of an *α*,*β*-unsaturated carbonyl carbon. There was also a correlation of H-13 with C-7 (δ44.7). The doublet methyl proton (δ1.08, H-14) exhibited correlations with carbon signals at δ29.6 (C-10), 44.1 (C-9) and C-1 (48.7). Similarly, singlet methyl proton (H-15) was correlated with C-5 (δ50.0), C-6 (δ35.2) and C-4 (δ221.2) which indicated its position. The data generated for the compound elucidated was found consistent with the literature values of that of graveolide (**131**) (Table 11). Graveolide was reported from *Inula graveolens* (DNP).

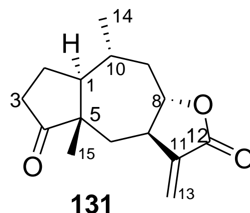


Table 11. The ^{1}H- and ^{13}C-NMR data of compound **131** and the data reported in the literature for graveolide ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz) **[114, 115]**

C No	NMR data of 131 δH	δC	NMR data of graveolide δH	δC
1	1.94 (1H,*m*)	48.7	1.92(1H,m)	48.7
2	2.10 (1H,*m*), 1.63 (1H,*m*)	24.1	2.10 (1H, m), 1.61 (1H, m)	24.1
3	1.52 (1H,m),2.48 (1H,*m*)	34.5	1.51(1H, m), 2.50(1H, m)	34.5
4		221.2		222.5
5		50.0		50.0
6	2.17 (1H,*m*), 2.48 (1H,*m*)	35.2	2.18 (1H, m), 2.47(1H, m)	35.2
7	2.81 (1H, *qt, J*= 12.4, 9.6, 3.6, 3.2)	44.7	2.81 (1H, m)	44.7
8	4.28(1H,*ddd,J*=12.0,9.2,3.2 Hz)	80.8	4.28(1H, ddd, *J*=11.6,8.8,2.8 Hz)	80.8
9	1.42 (1H,*m*),2.48 (1H,*m*)	44.1	1.42 (1H, m), 2.43 (1H, m)	44.1
10	1.94 (1H,*m*)	29.6	1.95(1H, m)	29.6
11		140.2		140.3
12		169.9		169.8
13	6.18 (1H,*d*, *J* = 3.6 Hz)	120.1	6.17(1H,d, *J*=3.2Hz)	120.0
13’	5.51 (1H,*d*, *J*= 3.2 Hz)		5.51(1H,d, *J*=3.2Hz)	
14	1.08 (3H,*d, J* = 6 *Hz*)	20.0	1.09(3H, d,*J*=6.0Hz)	20.0
15	1.04 (3H,*s*)	22.0	1.04(3H, s)	22.0

Assignments were made on the basis of COSY, HSQC and HMBC correlations

Compound 174

Compound **174** was isolated, together with compound **131**, as colorless needles after recrystallization from diethyl ether (Rf 0.39 in EtOAc:Hex (1:1); mp 77-78°C). The IR spectrum demonstrated the presence of ketonic C=O (1706 cm^{-1}), lactonic C=0 (1756 cm^{-1}), aliphatic C-H (2979/2943 cm^{-1}) and lactonic C=C (1644 cm^{-1}) suggesting the presence of isolated ketone and α, β-unsaturated γ-lactone functionalities in the compound. The ^{1}H NMR spectrum indicated the presence of two singlet at δ 1.10 (H-14) and δ 2.17 (H-15) due to methyl groups attached to quaternary carbons. Oxygenated methine proton signal at δ4.79 (*ddd*, *J*=14.8, 8.8, 6.0 Hz), methine proton signal at δ3.17 and exocyclic methylene proton signals (characteristic doublets at δ5.56 (*J*=2.4Hz) and 6.25 (*J*=2.8Hz)) were also observed (Fig.26). The spectrum also showed proton signals (minor ones) of compound **131**.

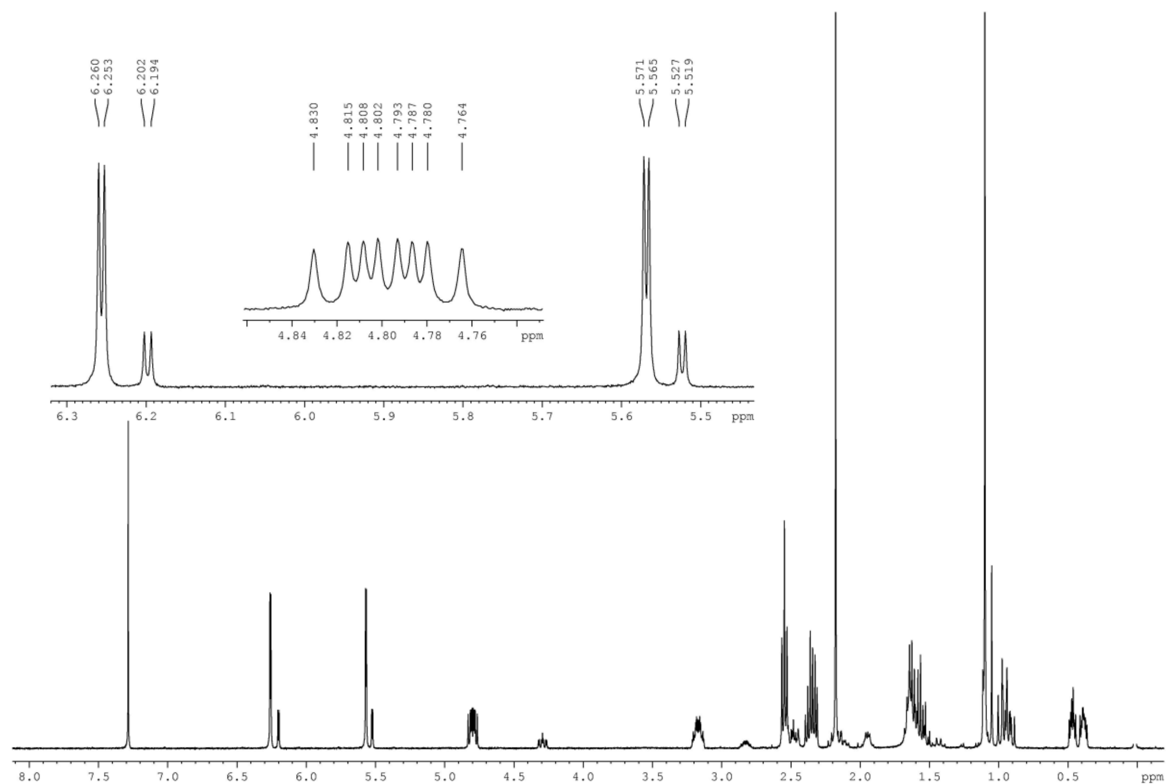


Fig 26. Expanded ^{1}H NMR spectrum of compound **174**

The triplet at δ2.53 (H-3) showed the presence of neighboring methylene group. Cyclopropane ring protons were also witnessed from the proton signals at δ 0.46 (1H, m) and 0.38 (1H, m). The ^{13}C NMR spectrum indicated methyl carbon signals at δ18.2 and 30.1, and oxymethine at δ75.6. The signals at δ17.2 and 22.9 can be attributed to cyclopropane ring carbons. The carbon signals at δ122.6 and 139.0 were attributable to a sesquiterpene lactone with exocyclic double bond. The carbon resonances at δ170.5 and 208.7 are due to a lactone carbonyl and a ketonic carbonyl carbon, respectively.

Two multiplets at δ0.46 (H-1) and 0.38 (H-5) showing ^{1}H-^{1}H COSY correlations between themselves and HMBC correlations with C-14 (δ18.2) were indicative of a three-membered ring moiety. The ^{13}C signals at δ208.7 and 30.1 (C-15) along with the ^{1}H signal at δ2.17 (3H, s,H-15) suggested the presence of an acetyl group which is attached to C-3 as inferred from the HMBC correlations of H-3 and H-15 with C-4 (δ208.7). ^{1}H-^{1}H COSY correlations of H-7 (δ3.17) with H-6 (δ0.95 and 2.35), H-8 (δ4.79) and H-13 (δ5.56 and 6.25) on the one hand and correlations of H-8 with H-7 and H-9 (δ0.95 and

2.35) together with HMBC correlations of H-13 with C-7 (δ37.7), C-11 (139.0), C-12 (170.5) established the presence of exocyclic α-methylene-γ-lactone moiety in the molecule. The EI-MS analysis showed molecular ion peak at m/z 248 corresponding to a molecular formula of $C_{15}H_{20}O_3$. The overall data generated was in close agreement with the literature value of carabrone (**174**) (Table 12).

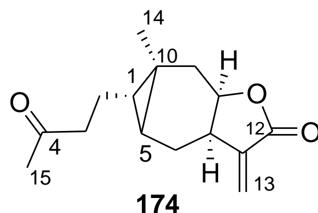


Table 12. The ^{1}H- and ^{13}C-NMR data of compound **174** and the data reported in the literature for carabrone ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz) [116-118]

C no	NMR data of 174		NMR data of carabrone
	^{1}H-NMR	^{13}C-NMR	^{13}C-NMR
1	0.46 (1H,m)	34.2	34.3
2	1.60 (2H,*m*)	23.3	23.3
3	2.53 (2H,*t,J=7.2*)	43.6	43.6
4	-	208.7	208.5
5	0.38 (1H,m)	22.9	22.9
6	0.95 (1H,*m*), 2.35(1H,*m*)	30.7	30.7
7	3.17 (1H,m)	37.7	37.8
8	4.79 (1H, ddd, J=6.0,8.8,14.8)	75.6	75.6
9	0.95 (1H,m), 2.35(1H,m)	37.3	37.3
10		17.2	17.2
11		139.0	139.0
12		170.5	170.4
13	5.56 (1H,*d*,*J*=2.4), 6.25(1H,d, *J*=2.8)	122.6	122.5
14	1.10(3H,*s*)	18.2	18.2
15	2.17 (3H,*s*)	30.1	30.0

Assignments were made on the basis of COSY, HSQC and HMBC correlations

Carabrone is a sesquiterpene lactone first isolated from the fruits of *Carpesium abrotanoides*. It was also isolated from *Inula grandis*. It showed cytotoxic, antibacterial and antitumor activities. It also exhibited antifungal activities *in vitro* and *in vivo* against *Botrytis cinerea*, *Colletotrichum lagenarium* and *Erysiphe graminis*. The ketonic carbonyl

at C-4 was reported to have a role in the activity of carabrone besides α-methylene-γ-butyrolactone [119, 120, DNP].

Compound 139

Compound **139** was isolated as white powder (Rf 0.33 in EtOAc:Hex (1:1); mp 116-118°C). Although the UV spectrum had no significant absorption, the IR spectrum showed absorption bands at 3406 cm^{-1} (-OH), 1744 cm^{-1} (C=O), 1769 cm^{-1} (lactonic C=O), 1644 cm^{-1} (lactonic C=C), and 2981/2869 cm^{-1} (C-H) indicating the presence of hydroxyl, ring ketone and α, β-unsaturated γ-lactone functional groups in the compound. The pattern of ^{1}H and ^{13}C NMR spectra were similar to those of compound **131**. The ^{1}HNMR spectrum showed three multiplets (δ1.51, 2.23, 2.47) due to methylene protons and three multiplets at δ1.85, 2.23 and 2.90 corresponding to methine protons. Two doublets at δ 6.23 and 6.01 (*J*=3.6 and 3.2 Hz) corresponding to protons of an exocyclic methylene group were also observed (Fig. 27). Two oxymethine proton signals were observed at δ4.40 (*ddd, J*=12.0, 10.0, 2.8 Hz) and δ4.04 (d, *J*=8.8 Hz). An OH proton signal was appeared at δ1.70 as broad singlet. The proton signals at δ1.04 (3H, *s*) and 1.11 (3H, *d, J* = 6.8 Hz) showed the presence of two methyl groups where the latter is bonded to a tertiary carbon. The ^{13}C NMR spectrum depicted 15 well-resolved carbon signals. The DEPT-135 spectrum revealed signals at δ19.1 (3H, *d, J*=6Hz) and 20.1 (3H, s) due to two methyl groups of which the former was bonded to tertiary carbon. It also showed five methine (δ30.2, 45.2, 52.0, 75.4, 76.0), four methylene (δ 24.6, 37.7, 44.1, 122.0) and four quaternary carbon (δ57.8, 138.9, 169.7, 220.8) resonances. The presence of α-methylene-γ-lactone moiety was evidenced by carbon signals at δ138.9 (C-11), 122.0 (C-13), 169.7 (C-12) and 76.0 (C-8). A carbon signal at δ220.8 also signified a ketonic carbonyl. The oxygenated carbon signal at δ75.4 showed the existence of one tertiary alcohol in addition to the lactone oxygen (δ 76.0) bonded to carbonyl. The LC-ESI-MS of the compound showed a molecular ion at m/z 265.14 for $[M+H]^{+}$ corresponding to the molecular formula $C_{15}H_{20}O_{4}$. The overall spectral data demonstrated a sesquiterpne lactone of pseudoguaianolide skeleton.

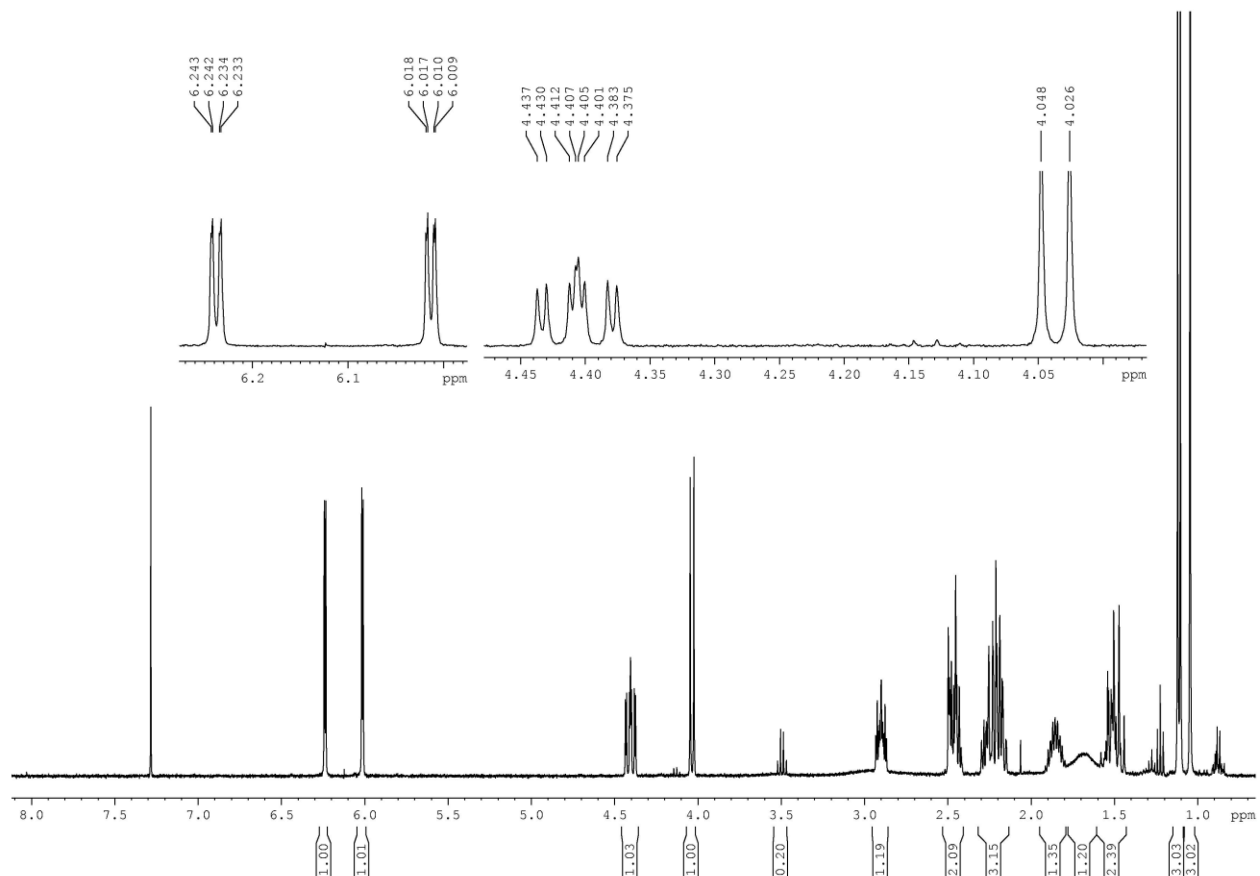
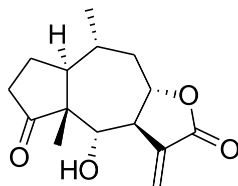


Fig 27. Expanded ^{1}H NMR spectrum of compound **139**

The ^{1}H and ^{13}C NMR spectroscopic data were assigned using ^{1}H-^{1}H COSY, HSQC and HMBC techniques. Correlations between H-13 (δ6.23 and 6.01) and H-7 (δ2.90), H-6 (δ4.04) and H-7, and H-14 (δ1.11) and H-10 (δ1.85) were observed in the ^{1}H-^{1}H COSY spectrum. Correlations were observed for H-7 with H-8 (δ4.40), H-6 and H-13, and inturn H-8 showed correlations with H-7 and H-9 (δ1.51 and 2.47). ^{1}H-^{1}H COSY correlations were also exhibited for H-10 with H-1 (δ2.23), H-9 and H-14 and in turn H-1 was correlated with H-2 (δ1.51 and 2.23) and H-10. HMBC correlations from H-6 to the carbons C-5 (δ57.8), C-7(δ52.0), C-11(δ138.9), C-15 (δ19.1), and from H-15 to C-1(δ45.2),C-4 (δ220.8), C-6 (δ75.4) and C-5 were evident. Correlations of H-7 with C-6, C-9, C-11, C-13 (δ122.0), and H-13 with C-7, C-11,C-12 (δ169.7) together with that of H-14 with C-1, C-9 (δ44.1), C-10 (δ30.2), and H-10 with C-14 (δ20.1) supported the proposed structure. All in all the data was compared and found consistent with the literature report for carpesiolin (**139**) (Table 13). The compound was isolated from

Carpesium abrotanoides and *Telekia speciosa***.** In addition it was demonstrated as potent cytotoxic agent [116].



139

Table 13. The ^{1}H- and ^{13}C-NMR data of compound **139** and the data reported in the literature for carpesiolin ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz) [121]

C no	NMR data of 139 δH	δC	NMR data of carpesiolin δH	δC
1	2.23 (1H, m)	45,2	2.15 (1H,m)	45.32
2	2.23(1H, m), 1.51(1H, m)	24.6	2.20 (1H, m), 1.53 (1H, m)	24.57
3	2.23(1H, m), 2.47(1H, m)	37.7	2.20(1H, m), 2.45(1H, m)	37.79
4		220.8		223.2
5		57.8		57.63
6	4.04 (1H, d,J=8.8Hz)	75.4	4.01 (1H, dd, *J*=9,3Hz)	75.35
7	2.90 (1H, m)	52.0	2.9 (1H, m)	52.35
8	4.40(1H, ddd,*J*=12,10,2.8Hz)	76.0	4.38(1H, ddd, *J*=11.5,10,3Hz)	76.14
9	1.51(1H, m), 2.47(1H, m)	44.1	1.48 (1H, m), 2.46 (1H, m)	44.28
10	1.85 (1H, m)	30.2	1.85(1H,m)	30.21
11		138.9		139.36
12		169.7		169.51
13	6.23 (1H, d,*J*=3.6Hz), 6.01 (1H, d,*J*=3.2Hz)	122.0	6.10(1H, d,*J*=3.5Hz), 5.98(1H, d,*J*=3Hz)	121.28
14	1.11 (3H, d, *J*=6.8Hz)	20.1	1.09(3H,d, *J*=6Hz)	20.02
15	1.04(3H, s)	19.1	1.04(3H, s)	18.99
OH	1.70 (1H,br s)	-	3.08 (1H,d, *J*=3Hz)	

Assignments were made on the basis of COSY, HSQC and HMBC correlations

Compounds 241 and 233

Compound **241** was obtained as colorless solid from the root of *Inula confertiflora*. The IR spectrum showed characteristic absorption bands at 3490 cm^{-1}(O-H), 2925/2870 cm^{-1} (C-H). Its ^{1}H NMR spectrum contained only one downfield doublet at *δ*3.75 (1H, *J*=2.8 Hz) indicating the presence of a proton on an oxygenated carbon (H-3). The spectrum also showed doublet at *δ* 0.90 (3H, d, *J*=6.8Hz, H-23) due to methyl protons attached to a tertiary carbon (C-4). The seven singlets at *δ* 0.87, 0.91, 0.94, 0.95, 0.97, 1.01 and 1.18 are attributed to methyl attached to quaternary carbons. The ^{13}C NMR spectrum showed 30 carbon resonances (Table 15) due to six quaternary, five methine, eleven

methylene and eight methyl groups. The presence of a hydroxyl group inferred from IR spectrum was evident in the ^{13}C NMR spectrum from the appearance of an oxymethine (C-3) carbon signal at δ72.8. The ^{1}H and ^{13}C NMR spectral data indicate that the compound belongs to the friedelane group of triterpenes. It was identified as friedelan-3β-ol (epifriedelanol) (**241**) by comparing the spectroscopic data with that reported in the literature [122,123]. The EIMS showed a molecular ion peak at m/z 428 corresponding to the molecular formula $C_{30}H_{52}O$ in agreement with the structure **241**. Friedelan-3β-ol (**241**) is widespread in plants such as *Aster tataricus* (DNP).

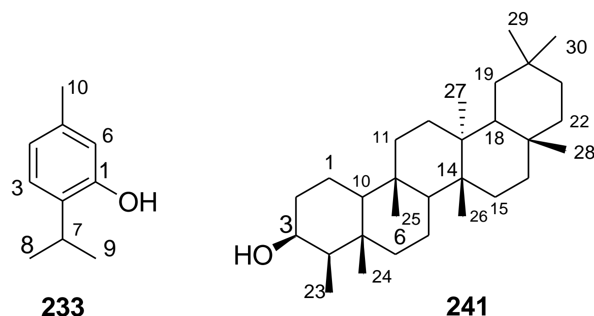


Table 14. The ^{13}C-NMR data of compound **241** and the data reported in the literature for epifriedelanol (CDCl$_3$, δ in ppm) [122, 123]

C no	Exp results δC	Lit results δC	C no	Exp results δC	Lit results δC
1	15.8	15.80	**16**	36.1	36.08
2	35.2	35.18	**17**	30.0	30.04
3	72.8	72.78	**18**	42.8	42.81
4	49.1	49.16	**19**	35.5	35.56
5	37.1	37.11	**20**	28.2	28.20
6	41.7	41.72	**21**	32.8	32.81
7	17.5	17.56	**22**	39.3	39.30
8	53.2	53.20	**23**	11.6	11.66
9	38.4	38.38	**24**	16.4	16.42
10	61.3	61.34	**25**	18.2	18.27
11	35.3	35.35	**26**	18.7	18.69
12	30.6	30.65	**27**	20.1	20.15
13	37.8	37.84	**28**	31.8	31.81
14	39.7	39.68	**29**	35.0	35.06
15	32.3	32.33	**30**	32.1	32.11

In ^{13}C NMR spectrum of another colorless solid isolated from the root contained 39 carbon signals of which the signal at δ22.7 was intense. In the ^{1}H and ^{13}C NMR spectra of the solid there were signals identical with compound **241**. GC-MS analysis of the isolated sample confirmed the presence of **241** and **233**. Thus, the remaining NMR

signals were compared with that of authentic thymol and found identical as discussed below. Aromatic methine protons appeared in ^{1}H NMR spectrum at δ 7.10 (1H, *d*, *J* =7.6 Hz, H-3), 6.75 (1H, *d*, *J* = 7.2Hz, H-4) and 6.60 (1H, br s, H-6). The methine proton signal at δ3.18 (1H, septet, H-7) showed its coupling with six neighbouring protons. Methyl proton signals were also observed at δ1.26 (6H, *d*, *J*=0.8 Hz) and 2.29 (3H, s). The ^{13}C NMR spectrum also showed three quaternary aromatic carbon signals at δ152.6(C-1), 131.3 (C-2) and 136.6 (C-5), methyl carbon signals at δ22.7 (C-8, 9), 20.9 (C-10), three aromatic methine carbon resonances at δ126.2 (C-3), 121.6 (C-4), 116.0 (C-6) and aliphatic methine carbon signal at δ26.7(C-7). The structures of compounds **233** and **241** were confirmed by comparison of their ^{1}H and ^{13}C NMR data with those reported in the literature [122,123].

Compound 245

Compound **245** was isolated as white crystals (Rf 0.44 in EtOAc:Hex (1:1); mp 149-150°C) from extracts of *I. confertiflora* root. Its molecular formula was determined as $C_{32}H_{52}O_2$ using EI-MS analysis (m/z 468 ($M^{+\cdot}$)). The IR spectrum exhibited absorption bands at 1728 cm^{-1}(C=O) and 2963/2850 cm^{-1} (C-H). The ^{1}H NMR spectrum ($CDCL_3$) showed the presence of olefinic methylene proton signals at δ 4.72 (1H,s) and 4.74 (1H,s), vinyl proton at δ5.14 (1H,m) and oxymethine proton at δ4.50 (1H,m). Two singlets at δ1.63 (H-26) and 1.71 (H-27) due to allylic methyl groups were also observed. Six additional singlets at δ1.27, 1.33, 0.89, 0.87, 0.99 and 2.05 corresponding to H-18, H-19, H-28, H-29, H-30 and acetyl methyl protons were also evident, respectively. The ^{13}C NMR spectrum ($CDCl_3$) showed thirty two resolved carbon signals and DEPT-135 spectrum identified them as eight methyl, seven quaternary, eleven methylene and six methine carbon signals. In the ^{13}C NMR spectrum seven singlets at δ16.3 (C-18), 15.7 (C-19), 25.7 (C-26), 17.7 (C-27), 28.0 (C-28), 15.9(C-29), 16.5 (C-30) were due to methyl groups. The spectrum also showed the presence of one terminal double bond resonating at δ152.7 (C-20) and 107.5 (C-21), and a substituted olefin at δ124.5 (C-24) and 131.4(C-25). Downfield oxymethine carbon signal (δ80.9) alongwith α-methyl (δ21.3) and carbonyl (δ171.0) signals showed the existence of acetyl group. Even though several inconsistencies in the assignments of the NMR chemical shifts were observed in

the literature the spectroscopic data was in close agreement wth reported values of dammara-20,24-dien-3-yl acetate (**245**) (Table 15). It was obtained from *Olearia paniculata* (DNP).

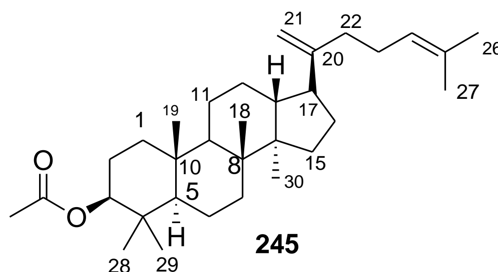


Table 15. The ^{13}C-NMR data of compound **245** and the data reported for dammara-20,24-dien-3-yl acetate ($CDCl_3$, δ in ppm, Mult) [124]

C no	Exp results δC	Lit results δC	C no	Exp results δC	Lit results δC
1	38.8	37.9	**17**	47.8	47.9
2	23.7	23.7	**18**	16.3	16.3
3	80.9	80.9	**19**	15.7	15.7
4	37.9	38.8	**20**	152.7	152.7
5	56.0	56.0	**21**	107.5	107.5
6	18.2	18.2	**22**	34.2	34.2
7	35.4	35.4	**23**	28.9	28.9
8	40.5	40.5	**24**	124.5	124.5
9	50.9	50.9	**25**	131.4	131.4
10	37.2	37.2	**26**	25.7	25.7
11	21.4	21.4	**27**	17.7	17.7
12	24.9	25.0	**28**	28.0	28.0
13	45.3	45.3	**29**	15.9	15.9
14	49.4	49.5	**30**	16.5	16.5
15	31.4	31.4	C=O	171.0	170.9
16	27.1	27.1	CH_3	21.3	21.3

Compound 107

Compound **107** was isolated as gum (Rf 0.25 in EtOAc:Hex (1:1)) from extracts of *I. confertiflora* flowers. Its IR spectrum demonstrated absorption bands at 3448 cm^{-1} (-O-H), 2949 cm^{-1} (-C-H), 1753 cm^{-1} (-C=O) and 1660 cm^{-1} (C=C). The ^{1}H NMR spectrum showed a signal at δ4.33 (H-8, *ddd*, *J*=6.0, 9.2, 12.0) due to oxymethine proton and doublets at δ6.23 (1H, *J*=3.6 Hz), 5.55 (1H, *J*=3.2Hz), 5.10 (1H,s) and 4.97(1H,s) attributable to two sets of exocyclic methylene groups showed the presence of α-methylene-γ-lactone moiety and terminal double bond at C-10 and C-14 (Fig 28).

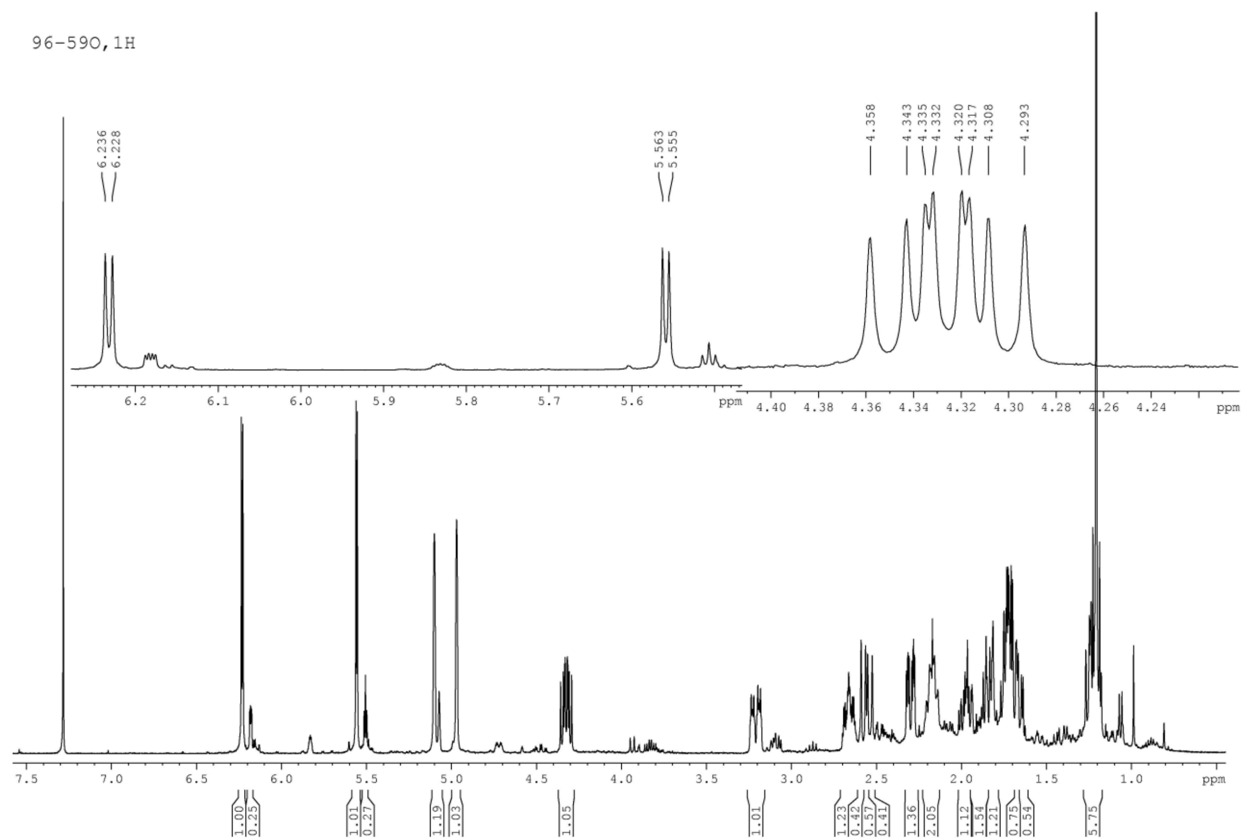


Fig 28. Expanded ^{1}H NMR spectrum of compound **107**

Only one singlet methyl proton signal was observed at δ1.22 and its coupling to a quaternary carbon was evident. In ^{13}C NMR spectrum fifteen carbons were observed from 24.00-171.00 ppm comprising six methylene, four methine and one methyl carbon signals. Methine carbon signals at δ45.4 (C-7) and δ82.4 (C-8) along with exocyclic double bond resonances (δ139.6, C-11 and 120.5, C-13) and that of lactone carbonyl (δ170.2, C-12) showed the presence of α-methylene-γ-lactone unit. Signals at δ111.7(C-14) and 146.6 (C-10) indicated additional exocyclic double bond. The quaternary carbon signal at δ80.4 exhibited an oxygenated carbon and this refered the alcohol function in support of IR data. The presence of methyl carbon (δ24.1) at the carbon where an alcohol function is bonded (C-4) was predictable from the ^{1}H and ^{13}C-NMR spectra analysis. The overall data indicated a guaianolide-type sesquiterpene lactone. This was also supported with ^{1}H-^{1}H COSY spectrum which showed correlations of H-13 (δ5.55 and 6.23) with H-7 (δ2.67), H-9 (δ2.56 and 3.20) with H-14 (δ4.97 and 5.10), H-8 (δ4.33) with H-7 and H-9, H-5 (δ1.68) with H-6 (δ1.21 and 2.31) and H-15 (δ1.22), and H-7 with

H-8 and H-13. The spectral data was found in accordance with literature report for inuviscolide (**107**) [125] (Table 16). It was also obtained from *Inula viscosa* and *Inula graveolens* (DNP).

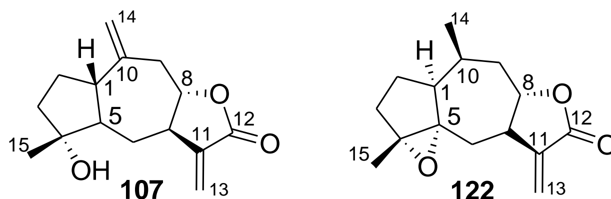


Table 16. The ^{13}C-NMR data of compound **107** and **122** and the data reported for Inuviscolide and 4α, 5α-epoxyinuviscolide ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz) [125]

C no	Exp results of 107 δC	Lit results of 107 δC	Exp results of 122 δC	Lit results of 122 δC
1	46.9	46.9	47.7	47.7
2	26.3	26.3	30.6	30.6
3	41.1	41.1	32.7	32.7
4	80.4	80.4	69.9	69.9
5	59.1	59.1	69.7	69.7
6	29.9	29.9	28.9	29.0
7	45.4	45.3	44.4	44.4
8	82.4	82.3	82.6	82.6
9	40.7	40.7	40.4	40.4
10	146.6	146.6	34.6	34.6
11	139.6	139.6	139.1	139.1
12	170.2	170.1	170.0	170.0
13	120.5	120.4	119.8	118.9
14	111.7	111.7	14.6	14.7
15	24.1	24.1	15.5	15.6

Compound 122

Compound **122** was also obtained as jelly material (Rf 0.42 in EtOAc:Hex (1:1)) from the extract of *I. confertiflora* flowers. The IR spectrum showed strong absorption bands at 2937 cm^{-1} (-C-H), 1765 cm^{-1} (-C=O) and 1656 cm^{-1} (C=C). In the ^{1}H NMR spectrum signals at δ4.05 (1H,*ddd, J*=12.0, 9.0, 6.0, 4.0Hz), δ2.99 (1H,m), 2.58 (1H,*d*,*J*=8.8 Hz) and 2.11(1H,m) showed methine protons of which the first was oxymethine. Two methyl proton signals were also observed at δ0.95 (3H,*d*, *J*=9.6 Hz) and 1.38 (3H,s) where the former was attached to a tertiary carbon. The presence of only two doublet proton signals at δ5.51 and 6.22 was an indicator for the occurrence of one set of exocyclic methylene group (Fig. 29). This was supported by the resonances of terminal double bond at δ139.1 and 119.8 in ^{13}C NMR spectrum. Together with signals at δ170.0 and

82.6, the data established the presence of α-methylene-γ-lactone moiety. From fifteen carbon signals showed in the ^{13}C NMR spectrum, four of them were identified as quaternary carbons including one lactone carbonyl and substituted terminal double bond using DEPT-135 spectrum. Quaternary carbon signals at δ69.7 and 69.9 suggested the existence of an epoxide ring. ^{1}H-^{1}H COSY correlations observed between H-13 (δ5.51 and 6.22) and H-7 (δ2.99), H-7 with H-6 (δ1.27 and1.86), H-8 (δ4.05) and H-13 indicated exocyclic α-methylene-γ-lactone moiety. Comparison of the spectroscopic data with the reported data showed that the compound is a guaianolide-type sesquiterpene lactone, namely, 4α, 5α-epoxyinuviscolide (**122**) [125] (Table 16).

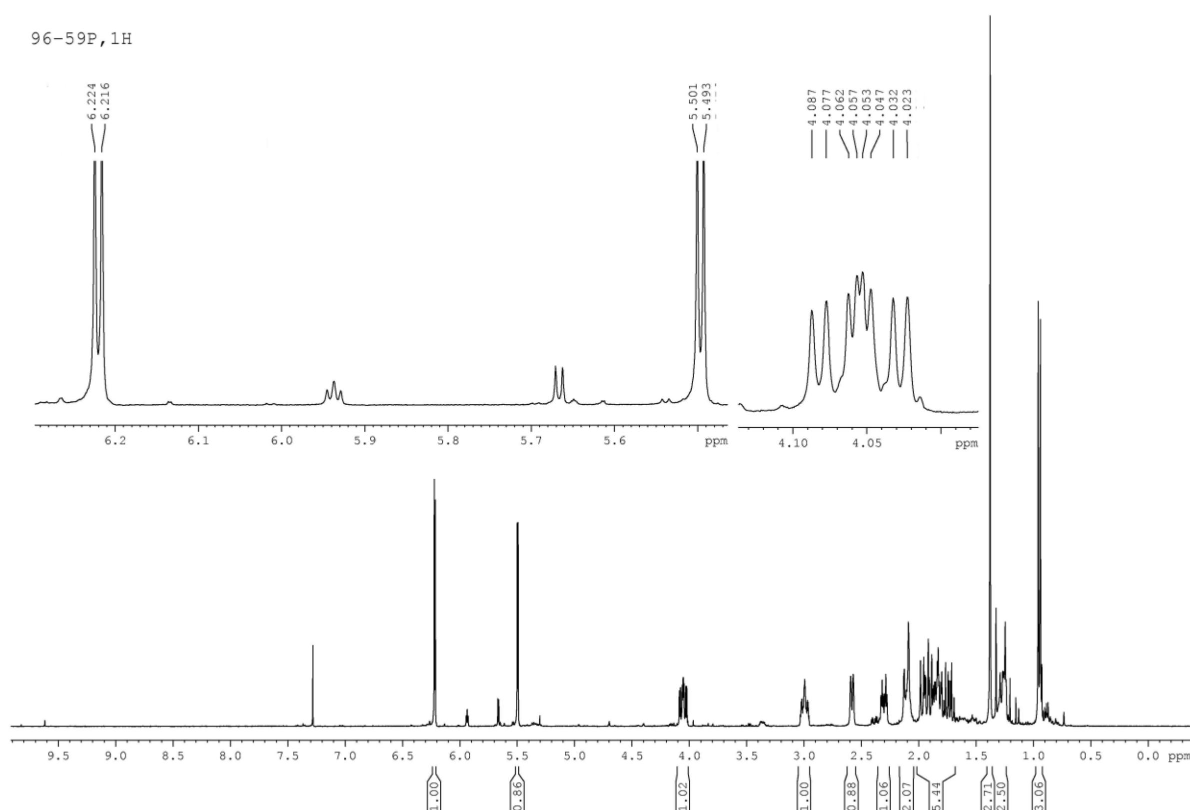


Fig 29. Expanded ^{1}H NMR spectrum of compound **122**

2.3.2 Identification of chemical constituents of essential oils from *I. confertiflora*

There was no previous report on chemical analysis of essential oils isolated from *I. confertiflora.* In this work, volatile oils were extracted from the root, flowers and leaves of the plant using Clevenger-type apparatus and subjected to GC-MS analysis to identify

the chemical components by comparing their recorded mass spectra with those stored in the mass spectral library (NIST) of the GC–MSD data system.

I. confertiflora root

Hydrodistillation of powdered root (50 g) resulted in 50 mg (0.1%) essential oil. GC-MS analysis of the volatile oil led to the identification of 6 components representing 93.01% and nine of them were found minor (Table 17, Fig 30). The root essential oil was characterized mainly by 2,5-Dimethoxy-4-isopropyltoluene (31.86%) (**479**), thymol (30.92%) (**233**) and 1-methoxy-4-(1-methylethyl) benzene (18.10%) (**481**).

Table 17. Chemical composition of essential oil from *I. confertiflora* root

Peak no	Compound name	Chemical structure	Retention time (min)	% comp	Quality
1	2-Methyl-3-phenyl-2-propenal	**477**	8.231	02.50	70%
2	3-Methyl-4-isopropylphenol	**478**	8.841	04.93	93%
3	Thymol	**233**	8.904	30.92	70%
5	2,5-Dimethoxy-4-isopropyltoluene	**479**	10.115	31.86	91%
6	1,4-Dimethoxy-2-methyl-5(prop-1-en-2-yl) benzene	**480**	10.311	04.70	94%
8	1-Methoxy-4-(1-methylethyl) benzene	**481**	10.673	18.10	76%

I. confertiflora flowers

Hydrodistilation of dried flowers (50 g) gave 70 mg (0.14%) essential oil. GC-MS analysis showed the presence of many components, of which five of them represented more than 61% of the oil. The major components of the oil were identified as 2,5-dimethoxy-4-isopropyltoluene (24.95%) (**479**), thymyl isobutyrate (16.53%) **234** and 6-methoxy thymylisobutyrate (11.53%)(**483**) (Table 18, Fig 30).

Table 18. Chemical composition of essential oil from *I. confertiflora* flower

Peak no	Compound name	Chemical structure	Retention time min)	% comp	Quality
3	2,5-Dimethoxy-4-isopropyltoluene	**479**	12.713	24.95	93%
4	1,4-Dimethoxy-2-methyl-5-(prop-1-en-2-yl)benzene	**480**	12.987	06.35	93%
5	Thymylisobutyrate	**234**	13.512	16.53	95%
6	2,4-Di-tert-butylphenol	**482**	13.839	03.74	97%
13	6-methoxy thymylisobutyrate	**483**	15.902	11.53	90%

I. confertiflora leaves

Although it was difficult to get volatile oil from the dried leaves of *I. confertiflora,* hydrodistillation of the fresh leaves (50 g) using Clevenger-type apparatus afforded 20µL essential oil (0.00004%). Analysis of this oil using GC-MS showed constituents qualitatively similar with that of the flowers (Table 19, Fig 30).

Table 19. Chemical composition of essential oil from *I. confertiflora* leaf

Peak no	Compound name	Chemical structure	Retention time min)	% comp	Quality
5	2,5-Dimethoxy-4-isopropyltoluene	**479**	12.712	9.97	96%
6	1,4-Dimethoxy-2-methyl-5-(prop-1-en-2-yl)benzene	**480**	12.987	3.27	-
7	Thymylisobutyrate	**234**	13.512	6.63	-
8	2,4-Di-tert-butylphenol	**482**	13.839	3.31	-
14	6-methoxy thymolisobutyrate	**483**	15.906	8.50	-

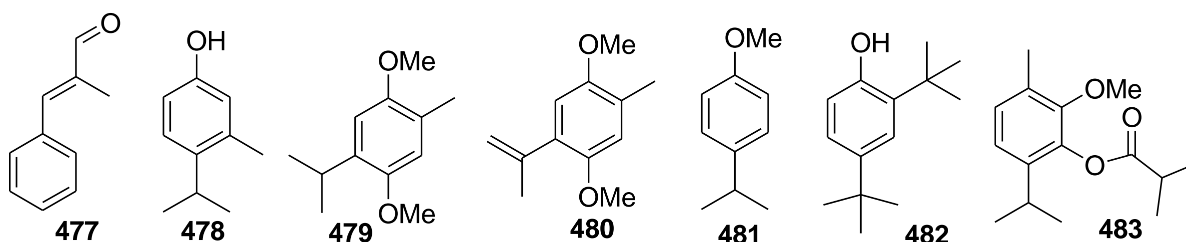


Fig. 30. Chemical components found in essential oils isolated from *I. confertiflora*

2.3.3 Characterization of dehydrocostus lactone isolated from *E. Kebericho* root and its modified analogues

Ethanol extract (4 g) of *Echinops kebericho* root, collected from Addis Ababa, was screened for its antifungal activity and showed encouraging inhibitory activity ((%GI=73/80) against *F. spp*. / *P. infestans,* respectively. It was subjected to bioassay guided chemical study. The hexane+$CHCl_3$ (1:1) fraction of the EtOH extract was found more active (%GI=76/76) than the methanol fraction (%GI =37/46) against fungi under study. This nonpolar fraction was subjected to column filtrations in solvent gradient and recrystallizations in hexane to afford dehydrocostus lactone (DHCL) (**108**) as major component. The *in vitro* antifungal activity of the compound was evaluated and found promising (%GI =78/82) in the control of *F. spp*. / *P. infestans,* respectively.

In order to understand the role of change of structure on its bioactivity modifications were made on DHCL. Hence, it was treated with I_2/benzene, m-CPBA/ DCM, KOH/ MeOH, $NaBH_4$/ MeOH and octylamine/ MeOH to afford compounds **427/433**, **430**, **484** and **485**, respectively where reactions with KOH/ MeOH and $NaBH_4$/ MeOH gave identical compounds. An attempt to transform DHCL using hydrogenation reaction in the presence of H_2/Pd-C was not successful. The structure of each compound was elucidated using spectral data as follows.

Compound 108

Compound **108** was obtained as white crystals (Rf 0.36 in EtOAc:Hex (1:1); mp 56-57°C) from *E. kebericho* root as the main component. The IR spectrum revealed the presence of C-H (3081/2956 cm^{-1}), C=O (1744 cm^{-1}) and C=C (1644cm^{-1}). The spectrum also described the presence of olefinic methylene C-H bonds and α-methylene lactone function. The ^{1}HNMR spectrum was integrating for eighteen hydrogens comprising seven methylene and four methine protons. Saturated methylene proton signals were observed at δ1.94 (2H, m, H-2), 2.52 (2H, m, H-3), 1.42 and 2.20 (2H, m, H-8) and 2.20 and 2.52 (2H, m, H-9). Two singlets at δ4.79 and 4.88 (H-14) and two doublets at δ5.04 (1H, *d*, *J* = 1.6 Hz) and 5.24 (1H, *d*, *J*= 2.0 Hz) (H-15) showed the existence of two sets of isolated exocyclic methylene protons. Two doublets at δ 5.48 (1H, *d*, *J* = 3.2 Hz) and 6.20 (1H, *d*, *J*= 3.6 Hz) (H-13) showed the presence of α-methylene-γ-lactone moiety (Fig 31). An oxymethine proton signal at δ3.94 (1H, *t*, *J*= 9.2 Hz, H-6) and three allylic ring proton signals at δ2.91 (3H, m, H-1, H-5, H-7) were also observed.

The ^{13}CNMR spectrum showed fifteen carbon signals. The DEPT-135 spectrum showed three quaternary and three methylene carbon resonances at δ139.7 (C-11) and 120.2 (C-13), δ149.2 (C-10) and 112.6 (C-14), and δ151.3 (C-4) and 109.5(C-15) which described three sets of terminal double bonds. In the ^{13}C NMR spectrum four saturated methylene carbon signals at δ30.3 (C-2), 32.6 (C-3), 30.9 (C-8), 36.3 (C-9) and four methine carbon resonances at δ47.6 (C-1), 45.1 (C-5), 85.3 (C-6) and 52.0 (C-7) were observed. The oxymethine carbon (85.3(C-6)) and carbonyl carbon (δ170.3 (C-12)) were part of α-methylene-γ-lactone unit as the ^{1}H-^{1}H COSY spectrum showed correlations of H-7 with H-13 and H-6.

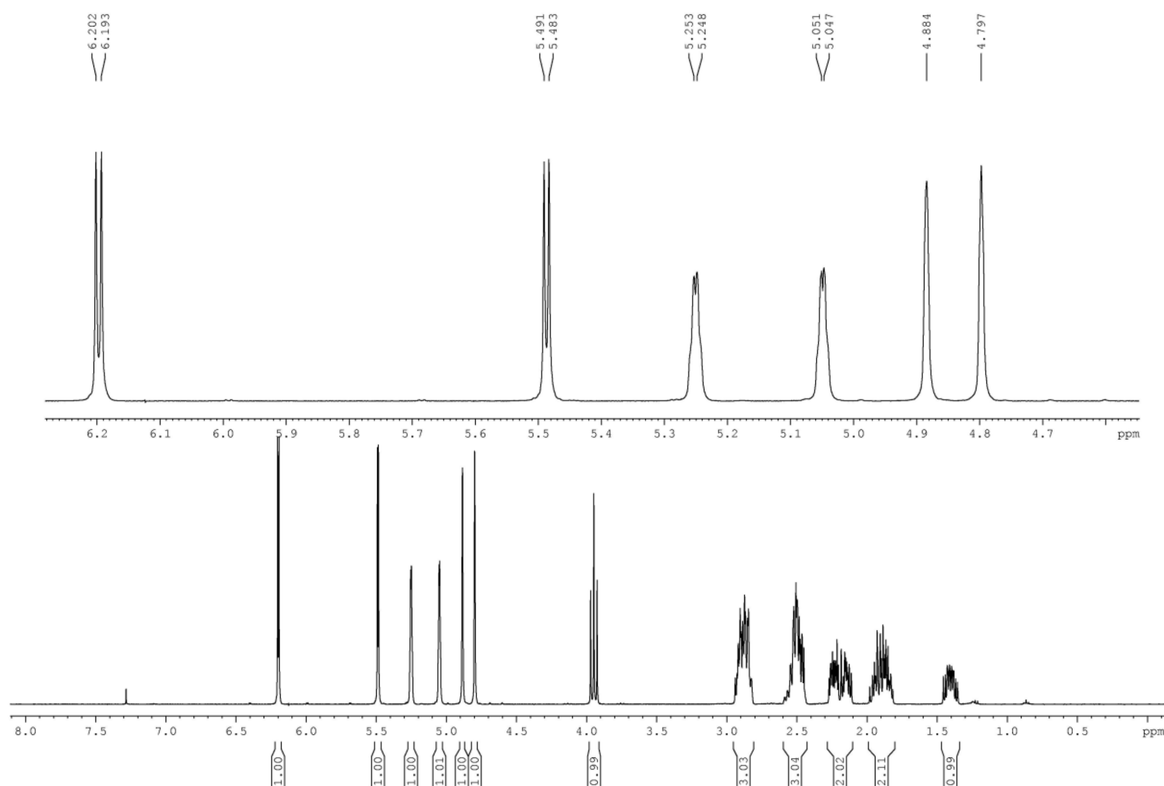


Fig 31. Expanded ^{1}H NMR spectrum of compound **108**

The LC-ESI-MS of **108** showed a molecular ion peak at m/z 230.96 (100%)) corresponding to the molecular formula $C_{15}H_{18}O_2$. The spectroscopic data was found consistent with the reported values for DHCL (**108**) (Table 20). It was also obtained from *Saussurea lappa*, *Veronica hirsuta* and *Zaluzania tribolia* (DNP).

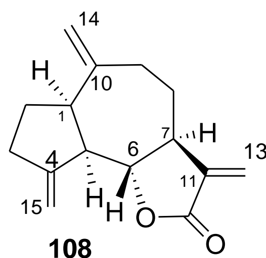


Table 20. The ^{13}C NMR data of **108** compared with reported data of DHCL ($CDCl_3$, δ in ppm, J in Hz) [84, 126, 127]

C no	NMR data of 108		NMR data of DHCL	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	2.91 (1H,*m*)	47.6	2.92 (1H,*m*)	47.5
2	1.94 (2H,*m*)	30.3	1.94 (1H,*m*), 1.88(1H,*m*)	30.2

3	2.52 (2H,*m*)	32.6	2.54 (2H,*m*)	32.5
4	-	151.3	-	151.2
5	2.91 (1H,*m*)	45.1	2.88 (1H,*m*)	45.0
6	3.94 (1H,*t*,J=9.2)	85.3	3.93 (1H, *t*, J=9.3)	85.1
7	2.91 (1H,*m*)	52.0	2.86(1H,*m*)	51.9
8	1.42(1H,*m*), 2.20 (1H,*m*)	30.9	1.42(1H,*m*), 2.24(1H,*m*)	30.8
9	2.20 (1H,*m*), 2.52 (1H,m)	36.3	2.17(1H,*m*), 2.48(1H,*m*)	36.2
10		149.2		149.1
11		139.7		139.7
12		170.3		170.2
13	6.20(1H,*d*,J=3.6), 5.48 (1H,*d*,J=3.2)	120.2	6.22 (1H,*d*, J=3.5), 5.39 (1H,*d*,J=3.2)	120.1
14	4.88(1H,*s*), 4.79 (1H,*s*)	112.6	4.90 (1H,*s*), 4.82 (1H,*s*)	112.5
15	5.24 (1H,*d*,J=2.0), 5.04 1H,*d*,J=1.6)	109.5	5.27 (1H,*d*,J=4.3), 5.07 (1H,*d*, J=4.2)	109.5

Compound 427/433

In order to synthesis isodehydrocostus lactone, DHCL was treated with molecular iodine in benzene. But mixtures of isomeric compounds **427/433** were obtained as brown oily material from the reaction. The IR spectrum showed the presence of C-H (2912 cm^{-1}) and C=O (1777 cm^{-1}) stretching vibrations. In ^{1}H NMR spectrum, the three singlets at *δ*1.67, 1.80 and 1.89 showed methyl protons. Signals of H-14 at *δ* 4.82 (1H,s) and 4.84 (1H,s), and signals of two sets of H-13 protons at *δ*5.34 (1H,*d*, *J*=2.8 Hz), 6.05 (1H, *d*, *J*=3.2Hz), 5.44 (1H, *d*, *J*=3.2Hz) and 6.14 (1H, *d*, *J*=3.6 Hz), respectively were observed. Two oxymethine proton signals at *δ*3.58 (1H, *t*, *J*=10 Hz) and 3.98 (1H, *t*, *J*=10 Hz) and a multiplet at *δ*5.48 integrating for two olefinic protons were also evident. The ^{1}H NMR spectrum showed the transformation of exocyclic double bonds in the starting material into endo-isomers where H-15 proton signals of the starting material were not seen. The ^{13}C NMR spectrum showed 29 carbon signals and it was assumed that one quaternary carbon signal was overlapped. In the DEPT-135 spectrum, eight signals at δ131.3 (C-4), 135.5 (C-4), 139.7 (C-11), 139.9 (C-11), 140.6 (C-10), 149.2 (C-10, C-1), 170.1 (C-12) and 170.4 (C-12) were quaternary carbons. The ^{13}C NMR spectrum also showed signals of three methyl carbons (*δ*16.8, 17.8, 23.2), two oxygenated carbons (*δ*85.2, 85.5), three exocyclic methylene carbons (*δ*113.0, 117.7, 119.9) and two olefinic methine carbons (*δ*126.2 (C-3) and 126.7 (C-3)) in support of the ^{1}H NMR data. In addition, six methylene carbon signals (δ26.0, 31.2, 34.0, 35.5, 37.2, 37.9) and five methine carbon resonances

(δ45.9, 47.4, 53.2, 56.0, 56.3) were shown. The overall ^{1}H NMR data (Table 21) showed similarity with reported values of isodehydrocostus lactone (**427)** and its isomer kauniolide (**433**) [84, 87]. **427** and **433** were isolated from *Saussurea lappa* as light green liquid and *Kaunia arbuscularis* as a gum, respectively (DNP).

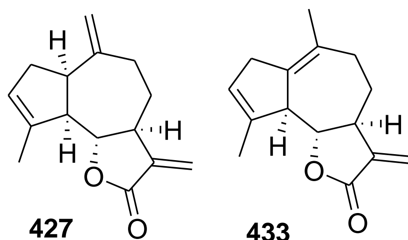


Table 21. ^{1}H NMR spectral data of **427** and **433** compared with lit. ($CDCl_3$, *δ* in ppm, *J* in Hz)

C no	NMR data of 427		NMR data of 433	
	Exp δH	**Lit δH**	**Exp δH**	**Lit δH**
1	2.80 (1H,m)	2.82 (1H,m)	-	-
2	2.41 (1H,m), 2.24 (1H,m)	2.46 (2H,m)	2.92 (2H,br s)	2.96 (2H,m)
3	5.48 (1H,m)	5.54(1H,s)	5.48 (1H,m)	5.52 (1H,m)
5	3.10 (1H,m)	3.15 (1H,m)	3.33 (1H,*d*,*J*=10,4)	3.30(1H)
6	3.98 (1H,*t*,*J*=10)	4.04 (1H,*t*,*J*=10)	3.58 (1H,t,*J*=10)	3.66 (1H)
7	2.80 (1H,m)	2.82 (1H,m)	2.80 (1H,m)	-
8	1.40 (1H,m), 2.08 (1H,m)	1.42 (1H,m), 2.12 (1H,m)	1.21 (1H,m), 2.08 (1H,m)	1.22 (2H)
9	2.24 (1H,m), 2.41 (1H,m)	2.25 (1H,m), 2.46 (1H,m)	2.24(1H,m), 2.31(1H,m)	-
13	5.44(1H,*d*,*J*=3.2), 6.14 (1H,*d*,*J*=3.6)	5.48 (1H,*d*,*J*=3), 6.20 (1H,*d*,*J*=3.5)	5.34 (1H,*d*,*J*=2.8), 6.05(1H,*d*,*J*=3.2)	-
14	4.82 (1H,s), 4.84 (1H,s)	4.87 (1H,s), 4.88 (1H,s	1.89(3H,s)	1.91 (3H)
15	1.80 (3H,s)	1.85 (3H,s)	1.67 (3H,s)	1.72(3H)

Compound 430

Compound **430** was obtained as white gum from the reaction of DHCL with m-CPBA in DCM. The IR spectrum showed absorption bands for C-H (2936 cm^{-1}) and C=O (1763 cm^{-1}). In ^{1}H NMR spectrum, olefinic exocyclic methylene proton signals at *δ* 4.95 and 4.99 (1H each, s) (C-14 (*δ*114.2)) and α-methylene proton signals at *δ*6.18 and 5.48 (1H each, d, *J*=3.2Hz) (C-13 (*δ*120.3)) were observed. A multiplet at *δ*4.06 showed oxymethine proton located at C-6 (*δ*82.0). Mutually coupled oxirane methylene doublets at *δ*2.86 and 3.38 (*J*=4.4 Hz) which were absent in the starting material indicated the formation of epoxide group at C-4 (*δ*66.5) and C-15 (*δ*50.8). The ^{13}C NMR also showed 15 carbon signals. In addition to above mentioned carbon signals three methine (*δ*46.9,

53.4, 46.3), four methylene (*δ*28.5, 31.5, 29.7, 33.3) and four quaternary carbons (*δ*66.5, 148.3, 139.5, 169.8) were displayed in the spectrum. Based on the data (Table 22) and literature reports [88] the structure of the compound was elucidated as 4,15-epoxydehydrocostus lactone (**430**).

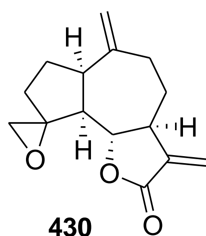


Table 22. ^{13}C NMR spectral data of **430** compared with literature ($CDCl_3$, *δ* in ppm)

C no	Exp results δC	Lit results δC	C no	Exp results δC	Lit results δC
1	46.9	46.8	**9**	33.3	33.1
2	28.5	28.4	**10**	148.3	148.1
3	31.5	31.4	**11**	139.5	139.3
4	66.5	66.4	**12**	169.8	169.8
5	53.4	53.2	**13**	120.3	120.2
6	82.0	81.8	**14**	114.2	114.1
7	46.3	45.8	**15**	50.8	50.1
8	29.7	29.8			

Compound 484

Compound **484** was synthesized as black jelly material from DHCL through alkaline hydrolysis using KOH/MeOH. The IR spectrum showed bond vibrations of C-H (2912 cm^{-1}) and C=O (1765 cm^{-1}). The ^{1}H NMR spectrum, integrating for twenty two hydrogens, showed the presence of only two sets of olefinic exocyclic methylene protons corresponding to H-14 (*δ*4.70, 4.80) and H-15 (*δ*4.96, 5.12) (Fig. 32).

Opening of the lactone ring and formation of a product containing methoxy proton signal at *δ*3.30, a new oxygenated methylene proton at *δ*3.61 (H-13) and methine proton at δ 2.37(H-11) were evident. The ^{13}C NMR spectrum also supported the above data where representative carbon signals were showed at *δ*176.07 (lactone C=O, C-12), 59.20 (-OMe), 68.87 ($-CH_2O$, C-13), 47.87 (CH, C-11) and exocyclic olefinic methylene carbon signals at *δ*111.66 (C-14) and 108.96 (C-15).

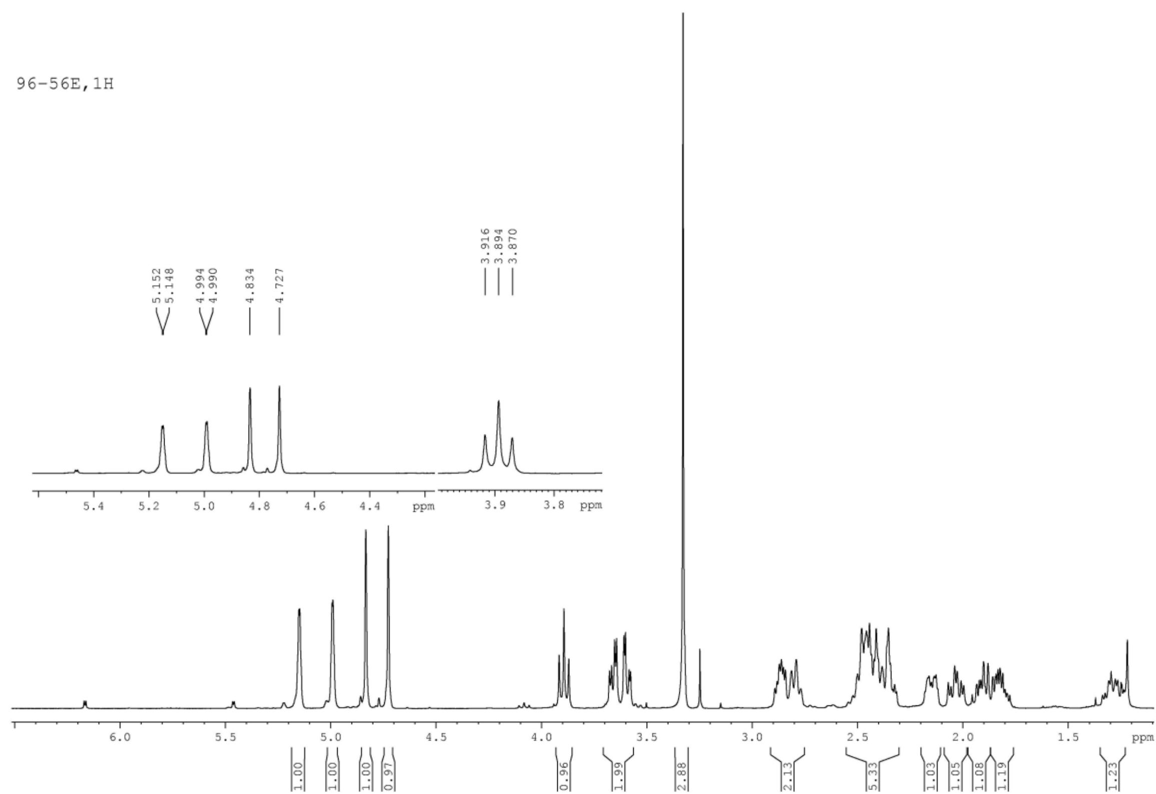
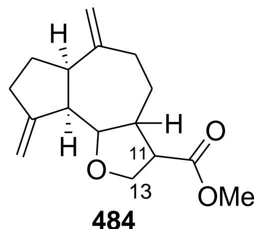


Fig 32. Expanded ^{1}H NMR spectrum of compound **484**

The ^{13}C NMR spectrum showed well resolved sixteen carbon signals comprising seven methylene, five methine, one methyl and three quaternary carbons as DEPT-135 described. In addition to the ester carbonyl carbon (C-12) quaternary carbon signals were observed at δ151.8 (C-4) and 150.0 (C-10). In ^{1}H-^{1}H COSY spectrum a signal at δ2.39 (H-7) showed correlations with δ3.86 (H-6), 2.37 (H-11) and 2.13 (H-8). H-11 was also correlated with δ3.61 (H-13). This data was confirmed by HMBC correlations of H-7 with δ85.5 (C-6), 32.5 (C-8) and 47.9 (C-11), and that of H-11 with δ176.1 (C-12), 68.9 (C-13) and 43.9 (C-7). The EIMS of **484** showed molecular ion peak at m/z 262.0 for $C_{16}H_{22}O_3$.

The structure of the compound synthesized was established as **484**. According to my knowdlege this structure was not reported in the literature. In order to transform DHCL into its α-methyl analogue (**429**) it was treated with $NaBH_4$/ MeOH. But the reaction product was isolated after work up as jelly material and it's IR, ^{1}H NMR and ^{13}C NMR spectra generated were found similar with that of **484**.



484

Compound 485

Compound **485** was obtained as yellow solid (mp 120-123°C) from the reaction of DHCL with octyl amine in MeOH. The IR spectrum of the compound showed strong absorption bands at 3398 cm^{-1} (N-H) 3086 cm^{-1} (=CH_2), 2866/ 2936 cm^{-1} (C-H) and 1764 cm^{-1} (C=O). The ^{1}H NMR spectrum showed signals at δ4.78 (1H,s), 4.85 (1H,s), 4.99 (1H,s) and 5.18 (1H,s) due to two sets of exocyclic methylene protons, respectively (Fig 33).

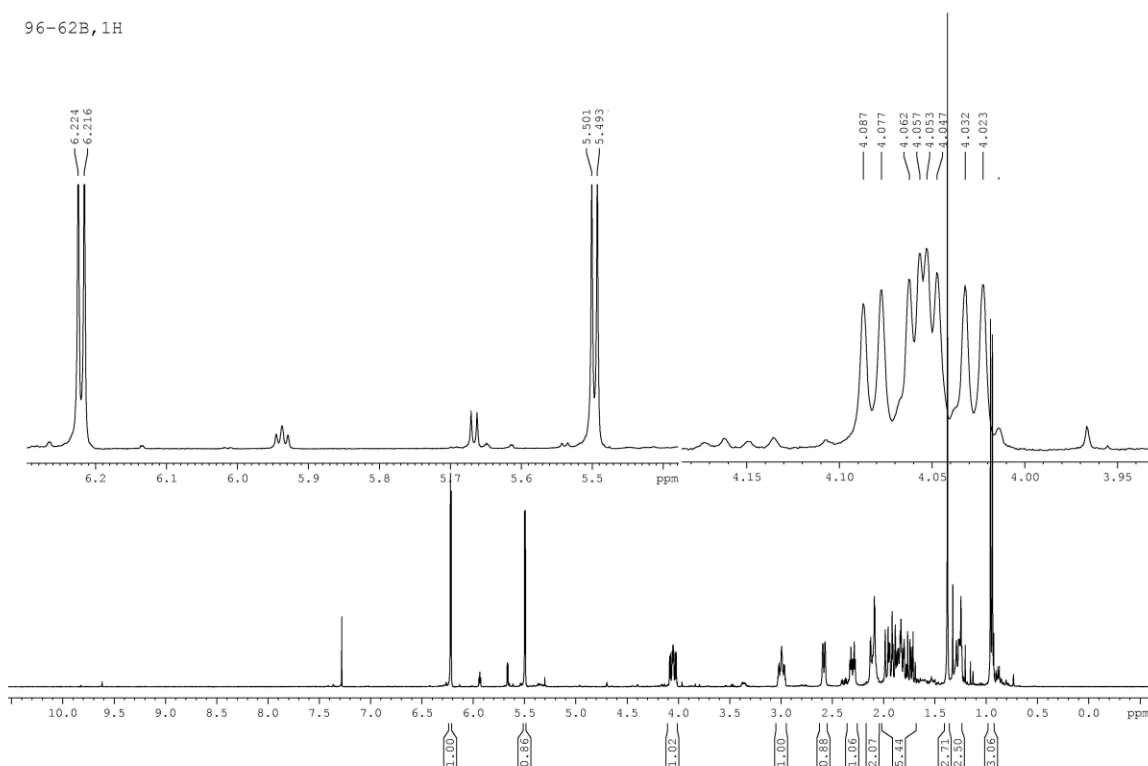
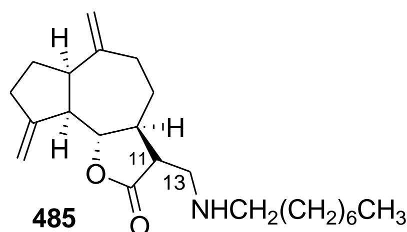


Fig 33. Expanded ^{1}H NMR spectrum of compound **485**

The terminal double bond conjugated to lactone carbonyl in the starting material was not observed. A new doublet at δ3.05 (2H, J=6 Hz) and a multiplet at δ2.82 (1H) were found and assignable to H-13 and H-11, respectively. The spectrum also showed oxymethine proton signal at δ3.98 (1H, *t*, 9.2 Hz), characteristic terminal methyl proton signal at

*δ*0.85 (3H, *t,* 6.8 Hz) and ten overlapped long chain methylene proton signals at *δ*1.23. A broad singlet at *δ*6.48 and two methylene proton signals at δ1.65 and 2.82 were observed and the former has no any correlations. The ^{13}C NMR spectrum demonstrated well resolved 23 carbon signals and the DEPT-135 spectrum counted them as one methyl, fourteen methylene, five methine and three quaternary carbons. Two terminal double bond carbon signals were showed at *δ*151.7 (C-4), 109.0 (C-15), 149.6 (C-10) and 111.9 (C-14) where as the exocyclic double bond conjugated to the carbonyl carbon was not indicated. All these data confirmed the addition of octylamine to the starting material. Carbon signals at δ86.3 (-CHO-, C-6), 45.4 (CH, C-7), 45.5(CH, C-11), 47.0 (-CH_2NH, C-13) and 177.6 (C=O, C-12) showed substituted furanone as established by ^{1}H-^{1}H COSY and HMBC experiments. In ^{1}H-^{1}H COSY spectrum correlations were observed for H-6 (δ 3.98) with H-5 (δ2.82) and H-7 (δ 2.26), H-7 with H-6, H-8 (δ1.38) and H-11(δ2.82), and H-11 with H-7 and H-13 (δ3.05). The correlations of H-6 with C-5 (δ51.8) and C-7 (δ45.4), H-7 with C-6 (δ86.3), C-8 (δ32.4) and C-11(δ45.5) and H-11 with C-7, C-12 (δ177.6) and C-13 (δ47.0) were observed in its HMBC spectrum. The EIMS of **485** revealed a molecular ion peak at m/z 359.0 corresponding to the molecular formula $C_{23}H_{37}NO_2$. The structure of the compound was elucidated as **485** and it was not reported in the literature.



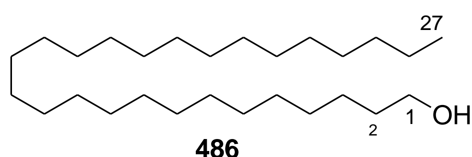
2.3.4. Characterization of compounds isolated from *Artemisia annua* leaf

Artemisia annua leaf was collected from ALNAP garden (AAU). Ground leaves (100 g) were extracted with ethanol (95%) to afford 15 g crude extract. It was fractionated into nonpolar (10 g) and polar parts (5 g). About 8 g of the nonpolar fraction was applied on top of column packed with silica gel (70-230 mesh) and gave three pure compounds, namely, 1-heptacosanol (**486**), artemisinin (**445**) and scopoletin (**404**) through several column fractionations and recrystallizations. In addition, the presence of artemisinic acid (**447**) and dihydroartemisinic acid (**448**) were identified from the same plant material. The

structures of the compounds isolated were elucidated using spectroscopic data and comparison with previous data in the literature.

Compound 486

Compound **486** was isolated as white powder (Rf 0.50 in EtOAc:Hex (1:1); mp 73-75°C) from leaf extracts of *A. annua*. The UV-Vis spectrum (in $CHCl_3$) showed no absorption maxima in the region. The IR spectrum displayed absorption bands at 3336 cm^{-1} (-OH) and 2923/2837 cm^{-1} (C-H). The ^{1}H-NMR spectrum showed a triplet at δ 3.66 (J = 6.4 Hz) assigned to methylene protons on oxygenated carbon adjacent to methylene proton. The quintet at δ1.60 integrated for two protons was due to methylene protons on carbon flanked between two methylene groups. A proton resonance at δ1.86 showed the presence of alcohol function. A broad intense singlet at δ1.32 (44H) is characteristic signal for many overlapping methylene protons which was supported by appearance of an intense carbon signal at δ 29.6 in the ^{13}C-NMR spectrum. An up field triplet at δ 0.89 (3H, J = 6.7 Hz) was an evidence for the presence of terminal methyl group closer to methylene protons. In the ^{13}C-NMR spectrum the down field signal at δ 63.1 corresponds to an oxygenated aliphatic methylene carbon. The carbon resonance at δ 14.2 is characteristic signal for terminal methyl group. Thus, comparison of the NMR spectral data of compound **486** with the reported value for heptacosan-1-ol was in close agreement (Table 23). It was isolated from *Citrullus colocynthis*, *Buddleja crispa* and *Crataegus monogyna* (DNP).



486

Table 23. The ^{1}H and ^{13}C-NMR spectral data for **486** compared with literature report ($CDCl_3$, δ in ppm, J in Hz) [113]

NMR data of 486		NMR data of 1-Heptacosanol	
^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
3.66 (2H, *t*, J = 6.4 Hz, H-1)	63.1	3.64 (2H, *t*, J = 6.3 Hz, H-1)	63.1 (C-1)
1.58 (2H, *m*, H-2)	32.8	1.57 (2H, *m*, H-2)	32.8 (C-2)
1.30(48H, *br s*, H-3 to H-26)	31.9	1.25(48H, *br s*, H-3 to H-26)	31.9 (C-3)
	30.0-29.4		29.7-29.3 (C4-24)
	25.8		25.7 (C-25)
	22.7		22.6 (C-26)
0.90(3H, t, J = 6.7Hz, H-27)	14.2	0.88 (3H, *t*, J = 6.6 Hz, H-27)	14.1(C-27)

Compound 445

Compound **445** was isolated as colorless needles (Rf 0.48 in EtOAc:Hex (1:1); mp 141-142°C) from leaf extracts of *A. annua.* The UV-Vis spectrum showed no absorption maxima. Strong IR absorption bands were observed at 2974/ 2849 cm^{-1} (C-H) and 1739 cm^{-1} (C=O). ^{1}H NMR spectrum was integrating for twenty two hydrogens. Signals at *δ*1.23 (3H, *d*, *J*=7.2Hz), 1.00 (3H, *d*, *J*=6.4Hz) and 1.45 (3H, s) were observed and assigned to methyl protons corresponding to H-13, H-14 and H-15, respectively. Two of them were found as doublet which showed their presence at tertiary carbon. In the spectrum four multiplets at *δ*1.40 (H-1), 1.43 (H-10), 1.78 (H-7) and 3.40 (H-11) due to methine protons and singlet at *δ*5.89 (H-5) owing to oxymethine proton were observed. Methylene protons were also evident as multiplet at δ1.48 and 2.05 (H-2), 2.08 and 2.43 (H-3), 1.07 and 1.90 (H-8) and 1.07 and 1.78 (H-9). There was no exocyclic double bond in the molecule.

The ^{13}C NMR spectrum showed fifteen carbon signals comprising three methyl, four methylene, five methine and three quaternary carbons as identified by its DEPT-135 spectrum. One of the methyl carbon signals found deshielded and singlet (*δ*25.2) representing its bonding closer to hetroatom. The other methyl carbons resonated at *δ*12.6 and 19.8 were bonded to tertiary carbon atoms. A tertiary carbon signal at *δ*93.7 (C-5), and quaternary carbon signals at *δ*105.4 (C-4) and 79.5 (C-6) were also distinguished as oxygenated. Lactone carbonyl signal was also showed at *δ*172.1(C-12) besides methine carbon signals at δ50.0 (C-1), 45.0 (C-7), 37.5 (C-10) and 32.9 (C-11). The data was comparable with reported values in the literature for artemisinin [99, 128] (Table 24). This sesquiterpene lactone has an endoperoxide oxygen bridge and it is also considered as the major active principle of the plant.

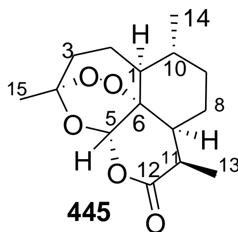


Table 24.^{1}H and ^{13}C NMR spectral data of Artemisinin **445** compared with literature ($CDCl_3$, δ in ppm, J in Hz) [99, 128]

C no	NMR data of 445		NMR data of Artemisinin	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	1.40 (1H,m)	50.0	1.37 (1H,m)	49.90
2	2.05 (1H,*m*),1.48 (1H,m)	24.9	2.01 (1H,*m*),1.47 (1H,m)	24.79
3	2.43 (1H,m),2.08(1H,m)	35.9	2.43(1H,*m*), 2.05(1H,*m*)	35.77
4		105.4		105.22
5	5.89 (1H, s)	93.7	5.87 (1H, s)	93.62
6		79.5		79.38
7	1.78 (1H,*m*)	45.0	1.75(1H,*m*)	44.80
8	1.90(1H,*m*),1.07 (1H,m)	23.4	1.87(1H,*m*), 1.12 (1H,m)	23.32
9	1.07 (1H,*m*),1.78(1H.m)	33.6	1.08 (1H, *m*), 1.79 (1H,*m*)	33.45
10	1.43(1H,*m*)	37.5	1.42 (1H,*m*),	37.42
11	3.40(1H,m)	32.9	3.40 (1H,*m*)	32.78
12		172.1		171.92
13	1.23(3H,*d*,J=7.2)	12.6	1.21 (3H,d,J=7.2)	12.47
14	1.00(3H,d,J=6.4)	19.8	0.99 (3H,d,J=6.4)	19.74
15	1.45(3H,*s*)	25.2	1.44(3H,s)	25.10

Compound 404

Compound **404** was isolated as yellow crystalline solid (Rf 0.41 in EtOAc:Hex (1:1); mp 198-200^{0}C) after recrystallizations in DCM. It showed blue fluorescence under UV light. It was UV-active and showed two absorption maxima at 295 nm and 340 nm. The IR spectrum showed absorption bands at 3338 cm^{-1} (O-H), 1702 cm^{-1} (C=O) and 1614/1564 cm^{-1} (benzene). The ^{1}H NMR spectrum (DMSO-d6) showed methoxy proton signal at δ3.81 (3H, s,-OMe). It also displayed singlets in aromatic region at δ7.21 (1H,s) and 6.78 (1H,s), and vinylic proton signals at δ 6.21(1H, *d*, *J*=9.6HZ) and 7.91 (1H, *d*, *J*=9.6HZ). The phenolic proton was not observed in ^{1}H NMR whereas the IR spectrum did it. The ^{13}C NMR spectrum showed ten carbon signals of which resonances at δ103.2, 111.0, 112.1, 145.7, 151.7 and 145.0 represented aromatic methine carbons and signal at δ56.5 showed methoxy carbon. Together with lactone carbonyl signal at δ161.2 and two olefinic carbon signals at δ110.0 and 145.0 the data showed a coumarin type skeleton. The overall data was found matching with literature values of scopoletin (**404**) (Table 25).

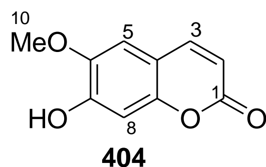
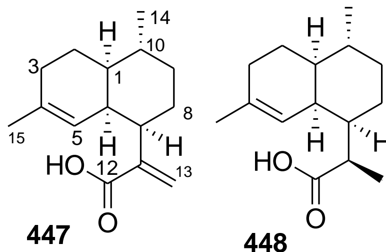


Table 25. ^{1}H and ^{13}C NMR data of **404** compared with literature value of scopoletin (DMSO-d6, δ in ppm, J in Hz) [129]

C no	NMR data of 404, DMSO-d6		NMR data of scopoletin, C_3D_6O	
	δH	δC	δH	δC
1	-	161.2	-	161.5
2	6.21 (1H,*d*,J= 9.6)	110.0	6.21 (1H,d,J=9.3)	107.6
3	7.91 (1H,*d*,J=9.6)	145.0	7.92 (1H,d,*J*=9.3)	143.4
4	-	111.0	-	111.5
5	7.21 (1H,s)	112.1	7.21 (1H,s)	113.4
6	-	145.7	-	144.1
7	-	151.7	-	150.3
8	6.78 (1H,s)	103.2	6.78 (1H,s)	103.2
9	-	150.0	-	149.8
10	3.81(3H,s, OCH_3)	56.5	3.87(3H,s,OCH_3)	56.5

Compound 447/448

The material was obtained as colorless oil. The IR spectrum showed strong absorptions at 2911 cm^{-1} (C-H) and 1714 cm^{-1} (C=O) and shoulder at 3500 cm^{-1} (-OH). The ^{1}H NMR spectrum showed four olefinic proton signals at δ4.98, 5.12, 5.57 and 6.47 of which the last two were due to exocyclic methylene protons as confirmed from DEPT-135 experiment. Methyl protons were also predictable from the spectrum at resonances δ0.90 (3H,m), 1.30 (6H,s), 1.60 (3H,m) and 1.18 (3H,m). In ^{13}C NMR spectrum thirty carbon signals were observed where signals at δ172.3 and 183.3 where assignable to carbonyl carbon of the acid functions in **447** and **448**, respectively.



Carbon signals at δ136.0 and 119.3 represented C4-C5 double bond of compound **448,** and signals at δ135.0, 120.2, 142.6 and 126.6 showed endo- and exocyclic double bonds of compound **447** located at C4-C5 and C11-C13, respectively. Five methyl

carbon signals were also observed at δ19.7, 23.7, 15.1, 19.7 and 23.8. Generally the NMR spectra of the material were found comparable with literature reports of artemisinic acid (**447**) and dihydroartemisinic acid (**448**) (Table 26).

Table 26. The ^{13}C-NMR data for **447/448** compared with literature report ($CDCl_3$, δ in ppm) [130, 131]

C no	Artemisinic acid		Dihydroartemisinic acid	
	Exp δC	Lit δC	Exp δC	Lit δC
1	41.4	41.3	43.6	43.6
2	25.6	25.5	34.0	35.2
3	26.4	26.3	27.4	27.3
4	135.0	134.9	136.0	135.9
5	120.2	120.1	119.3	119.2
6	37.9	37.8	42.1	42.1
7	42.0	42.0	41.7	41.7
8	25.9	25.9	26.6	26.5
9	35.2	35.2	25.8	25.7
10	27.6	27.5	27.7	27.6
11	142.6	142.5	36.4	36.3
12	172.3	172.3	183.3	183.0
13	126.6	126.6	15.1	15.0
14	19.7	19.9	19.7	19.6
15	23.7	23.7	23.8	23.7

2.3.5 GC-MS analysis of leaf essential oils from *Artemisia annua*, *Eucalyptus globulus* and *Thymus schimperi*

Artemisia annua: The leaves of *A. annua* were collected from ALNAP garden (AAU) and were dried. The powdered leaf (50 g) was hydrodistilled to afford 100 mg (0.2%) essential oil and subjected to GC-MS analysis. More than 80% of it was composed of camphor **443** [96-98] and it was followed by 2,5-dimethoxy-4-isopropyltoluene (2%)(Table 27).

Table 27. Chemical composition of Essential oil of *A. annua* leaf

Peak no	Compound name	Chemical structure	Retention time (min)	% comp	Quality
1	Camphor	**443**	8.837	80.90	98%
2	2,5-Dimethoxy-4-isopropyltoluene	**479**	12.710	2.30	95%

***Thymus schimperi**:* The dry leaves of *T. schimperi,* collected from Debre Sina, were ground and 50 g of it was hydrodistilled and gave 20 mg (0.04%) thyme oil. The chemical components of the essential oil were identified by GC-MS and in the course thymol

(72.5%) (**233**) and 1,8-cineol (16.39%) were found as major components. This composition was consistent with reported data [107] (Table 28).

Table 28. Chemical composition of leaf Essential oil of *T. schimperi*

Peak no	Compound name	Chemical structure	Retention time (min)	% comp	Quality
1	1,8-cineol	**397**	6.173	16.39%	-
2	Thymol	**233**	8.904	72.56%	-

***Eucalyptus globulus**:* The leaves of *E. globulus* were collected from ALNAP garden (AAU) and 50 g of the powdered material was hydrodistilled to afford eucalyptol oil of 30 mg (0.06%). The GC chromatogram of the oil showed the presence of 1,8-cineol (89.9%) (**397**) and β-pinene (5.92%)(**402**) as principal components [104, 105] (Table 29).

Table 29. Chemical composition of leaf Essential oil of *E. globulus*

Peak no	Compound name	Chemical structure	Retention time (min)	% comp	Quality
1	β-pinene	**402**	4.950	5.92%	-
2	1,8-cineol	**397**	6.173	89.9%	-

Thymol and 1, 8-cineol were reportedly antifungal agents [132]. They were supposed to play a role on the antifungal activity of *Thymus schimperi* and *Eucalyptus globulus* demonstrated in this study.

2.4 *In vitro* antifungal activity of isolated and modified compounds

Many plants were screened for their antifungal activity against *F.spp* and *P. infestans* and in the course *I. confertiflora*, *E. kebericho* and *A. annua* were selected for bioassay guided chemical investigations besides leaf essential oils from *E. globulus* and *T. schimperi*. The nonpolar fraction of their ethanol crude extract was found more active and in this chemical study different compounds were isolated and characterized. Modifications of DHCL into different derivatives were made to compare its antifungal activity with its analogues, and to get clue about the functional group responsible for its observed activity. In this *in vitro* antifungal assay eight isolated compounds and four modified natural products were included.

2.4.1 Antifungal activity of compounds isolated from *Echinops kebericho, Inula confertiflora* and *Artemisia annua*

DHCL (**108**), graveoilide (**131**), carpesiolin (**139**), carabrone (**174**), β-sitosterol (**306**), stigmasterol (**307**), artemisinin (**445**) and 1-heptacosanol (**486**) were subjected to *in vitro* antifungal activity test against *F.spp* and *P. infestans* at a concentration of 5 mg/mL using the optimized poisoned food bioassay technique. In the study **108**, **131**, **139**, **174** and **445** showed more than 50% growth inhibition percentages against the growth of fungi under study (Table 31). Compound **108** showed maximum growth inhibition potential (78%) whereas **174** demonstrated 71% inhibititory activity against *F. spp* and found comparable to the standard fungicide (68%). But the inhibitory activity of the other tested compounds found less potent than the standard. Compounds **108** (82%), **445** (75%) and **174** (73%) also showed better %GI in the control of *P. infestans* although they were weaker than the standard fungicide (88%) (Table 30). A free hydroxyl group at C-6 of **139** may help to increase its antifungal activity. The better active compounds contain exocyclic α, β- unsaturated lactone moiety in common except **445**. This functional group is known to undergo alkylation reaction with biological nucleophiles found in pathogens so as to control their cell division [60].

Table 30. *In vitro* antifungal activity (%GI) of isolated compounds

Compound	Mycelial growth (G) in mm (*F. spp.*)		Mycelial growth (G) in mm (*P.infestans*)	
	G $_{av}$	%GI	G $_{av}$	%GI
108	15.0 ± 1.5	78.0	08.0 ± 1.1	82.0
306	55.0 ± 6.5	21.0	37.0 ± 4.9	18.0
307	50.0 ± 3.0	28.0	34.0 ± 2.08	24.0
131	32.0 ± 3.6	54.0	19.0 ± 4.7	58.0
174	20.0 ± 2.6	71.0	12.0 ± 2.5	73.0
139	28.0 ± 2.5	60.0	16.0 ± 1.1	64.0
445	30.0 ± 3.6	57.0	11.0 ± 3.0	75.0
486	48.0 ± 3.7	31.0	22.0 ± 2.6	51.0
DMSO (aq) (Control)	70.0 ± 3.05	10.0	45.0 ± 1.4	02.0
Mancolaxyl (std)	22.0 ± 2.6	68.0	05.0 ± 1.0	88.0

Results are mean ± Relative standard deviation (%RSD), each experiments performed in triplicate

2.4.2 Comparison of antifungal activity of DHCL and its derivatives

DHCL was found the most active compound at a concentration of 5 mg/mL relatve to other tested phytochemicals in this study. To understand the effect of structural changes

and identify functional group responsible to its antifungal activity its modified analogues compound **427/433, 430, 484** and **485** were evaluated against *F.spp* and *P. infestans* and growth inhibition percentage was calculated and compared in Table 31.

Table 31. Fungal gowth inhibition percentages of DHCL and its analogues

Compound	**Mycelial growth (G) in mm (*F.spp.*)**		**Mycelial growth (G) in mm (*P. infestans*)**	
	G_{av}	%GI	G_{av}	%GI
108	13.0 ± 0.3	81.0	07.0 ± 0.5	84.0
427/433	12.0 ± 4.7	83.0	11.0 ± 3.7	75.0
430	19.0 ± 2.8	72.0	10.0 ± 1.5	78.0
484	23.0 ± 5.3	67.0	20.0 ± 4.4	55.0
485	27.0 ± 4.1	61.0	13.0 ± 3.5	71.0
DMSO (aq) control	70.0 ± 1.2	-	45.0 ± 0.6	-
Mancolaxyl (std)	27.0 ± 5	61.0	19.0 ± 3.2	57.0

Results are mean ± Relative standard deviation (%RSD), each experiments performed in triplicate

Generally DHCL was found still more potent (%GI > 80) than its analogues. Modified compounds namely compound **430** and the mixture (**427** and **433)** showed a higher inhibitory activity than the standard fungicide. Compound **430** exhibited %GI of greater than 70 against the test fungi. This is may be due to the presence of an epoxide group. Compound **484** demonstrated a growth inhibition percentage of 67/55 whereras **485** showed %GI of 61/71 against *F. spp*/ *P. infestans,* respectively (Table 32). Change of the lactone ring into tetrahydrofuran ring in **484** and alkylation of the *exo*-methylene group in **485** led to a decrease in antifungal activity suggesting that one of the structural requirments to the observed bioactivity of DHCL is the presence of α, β-unsaturated lactone function [60].

3. Experimental Part

The antifungal assay was conducted at Ambo Plant Protection Research Center/ Ethiopian Institute of Agricultural Research (APPRC/ EIAR (http://www.eiar.gov.et/) and the chemistry work was accomplished at Department of Chemistry, AAU.

3.1 Chemicals and equipment

All chemicals and solvents used were of analytical grade. Melting point was determined using Thomas Hoover capillary melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) and spraying with vanillin followed by heating with hot air gun. Column chromatography was performed using silica gel (70-230 mesh) Merck. Samples were applied on column by either adsorbing on silica gel or dissolving in appropriate solvent. Solvent was freed using rotavapor BUCHI, RE 111. The UV-Vis spectral measurements were done using UV-Vis on T 60 U spectrophotometer (PG instruments, UK) equipped with deuterium and tungsten lamps. NMR spectral measurements were done on Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr. Essential oil analysis was done with GC-MS (7820A GC system with Agilent technologies 5977E MSD, USA). LC-MS/EIMS was done at Korea Research Institute of Chemical Technology (KRICT) and Shanghai Institute of Materia Medica (SIMM).

Hood with laminar air flow and UV-radiation, Mancolaxyl®, Dimethylsulfoxide, Potato-Dextrose-Agar (PDA), Autoclave, Sterilized needle and cork borer, Incubator, Micropipette (1000 μL), Petridishes (90 mm) were used in antifungal assay.

3.2 List of plants collected for this study

Fifty seven different plant materials of various parts (leaf (Lf), root (Rt), fruit (Fr), seed (Sd), aerial part (Ap), flower (Fl)) were collected from Debre Birhan (D), Ankober (Ak), Wef Washa (W), Bure womberima (B), Ambo (A), ALNAP garden (Al) and local markets (M). Selection of plants collected was on the basis of availability, literature and interest of

collaborators (APPRC). Identification of plant materials was made by a professional botanist (AAU) and specimens of some plants were deposited at the National Herbarium of Ethiopia (AAU) (Table 32).

Table 32. List of plant species collected so far for screening

S.N	Species name	Family	Local name (place of coll)	Plant part	NDA No	Voucher no (NPC)
1	*Azadirachta indica*	Meliaceae	Neem (Eng) (M)	Lf,cake ,Sd, oil	218	S1211
2	*Hagenia abyssinica*	Rosaceae	Kosso (M)	Fl	272	S1203
3	*Melia azaderach*	Meliaceae	China berry (A)	Lf	1614	S1206
4	*Parthenium hysterophorus*	Asteraceae	Qenche arem (A)	Ap	3742	S1205
5	*Lantana camara*	Verbenaceae	Yewof qolo (A)	Ap	1550	S1204
6	*Salvia schimperi*	Lamiaceae	Debriq (D)	Ap,Sd	2890	S1144
7	*Croton macrostachyus*	Euphorbiaceae	Bisana (Al)	Lf	250	S1213
8	*Plantago lanceolata*	Plantaginaceae	Gorteb (B)	Lf	2718	S1141
9	*Inula confertiflora*	Asteraceae	Woynagift (Ak)	Ap, Rt, Fl	2531	S1152
10	*Leonotis ocymifolia*	Lamiaceae	Ras kimer (Ak)	Ap	2871	S1153
11	*Rumex nepalensis*	Polygonaceae	Tult (Al)	Rt	323	S1200
12	*Galiniera saxifrage*	Lamiaceae	Tota qula (W)	Ap	4104	S1187
13	*Olea europaea*	Oleaceae	Weyra (Al)	Lf	305	S1123
14	*Datura stramonium*	Solanaceae	Astenagir (Al)	Lf	256	S1214
15	*Uritica dioca*	Urticaceae	Samma (Al)	Lf	139	S1215
16	*Salvia officinale*	Lamiaceae	Sage (Al)	Ap	328	S1202
17	*Guizotia scabra*	Asteraceae	Mech (Al)	Ap	3711	S1150
18	*Galium simense*	Rubiaceae	Ashekit (Al)	Ap	4081	S1148
19	*Cynoglossum coeruleum*	Boraginaceae	Chegogot (Ak)	Ap	2584	S1161
20	*Maesa lanceolata*	Myrsinaceae	Qelewa (Ak)	Ap	3824	S1151
21	*Osyris quadripartita*	Santalaceae	Qeret (Ak)	Ap	1467	S1155
22	*Premna schimperi*	Lamiaceae	Chocho (Ak)	AP	6642	S1154
23	*Solanecio gigas*	Asteraceae	Dengoreza (Ak)	Ap	2545	S1188
24	*Balanites aegyptiaca*	Balanitaceae	Bedeno (M)	Fr	219	S1216
25	*Achyranthes aspera*	Amaranthaceae	Telenge (Al)	Ap	1881	S1201
26	*Vernonia amygdalina*	Asteraceae	Girawa (Al)	Lf	348	S1120
27	*Rumex nervosus*	Polygonaceae	Embacho (Al)	Ap	322	S1133
28	*Rumex abyssinicus*	Polygonaceae	Meqmeqo (Al)	Rt	321	S1147
29	*Ricinus communis*	Euphorbiaceae	Gulo (Al)	Ap	319	S1217
30	*Withania somnifera*	Solanaceae	Gizawa (M)	St	352	S1149
31	*Millettia ferruginea*	Fabaceae	Birbira (M)	Lf	294	S1218
32	*Olinia rochetiana*	Oliniaceae	Tife (Ak)	Ap	2006	S1162
33	*Allophylus abyssinicus*	Sapindaceae	Embis (Al)	Ap	1624	S1194
34	*Echinops kebericho*	Asteraceae	Kebericho (M)	Rt	83	S1219
35	*Warburgia ugandensis*	Canellaceae	Kanaf (S)	Lf	2069	S1207
36	*Artemisia annua*	Asteraceae	China chiqugn (Al)	Lf	212	S1220
37	*Verbascum sinaiticum*	Scrophulariaceae	Yeahiya joro (Al)	Lf, Fr	1254	S1199
38	*Moringa stenopetala*	Moringaceae	Shiferaw (M)	Oil	295	S1125
39	*Thymus schimperi*	Lamiaceae	Tosign (M)	Lf /EO	341	S1235

40	*Matricaria recutita*	Asteraceae	Chamomile (Al)	Fl	288	S1222
41	*Cucumis ficifolius*	Cucurbitaceae	Yemider embuay (Al)	Fr	2115	S1130
42	*Jatropha curcas*	Euphorbiaceae	Jatropha (M)	Oil	1250	S1223
43	*Eucalyptus globulus*	Myrtaceae	Nech bahirzaf (Al)	Lf/EO	261	S1233
44	*Chenopodium ambrosodes*	Chenopodiaceae	Amedmado (A)	Ap	1806	S1236
45	*Rhamnus prinoides*	Rhamnaceae	Gesho (Al)	Lf	318	S1225
46	*Syzygium guineense*	Myrtaceae	Doqma (M)	Lf	2202	S1121
47	*Kniphofia foliosa*	Asphodelaceae	Ashenda (Al)	Rt	1251	S1226
48	*Tagetes minuta*	Asteraceae	Gundanabir (Ak)	Ap	337	S1163
49	*Zehneria scabra*	Cucurbitaceae	Hareg resa (Al)	Ap	2103	S1128
50	*Sesbania sesban*	Fabaceae	Shewshewe (Al)	Ap	952	S1210
51	*Euclea divinorum*	Ebenaceae	Dedeho (M)	Fr	3814	S1127
52	*Thalictrum rhynchocarpum*	Ranunculaceae	Sirebezu (Ak)	Rt	369	S1157
53	*Lepidium sativum*	Brassicaceae	Feto (Al)	Ap	281	S1228
54	*Bersama abyssinica*	Melianthaceae	Azamr (Al)	Lf	1636	S1230
55	*Myrsine Africana*	Myrsinaceae	Kechemo (W)	Ap	297	S1229
56	*Cordia Africana*	Boraginaceae	Wanza (Al)	Lf	248	S1232
57	*Bidens macroptera*	Asteraceae	Adey Abeba (Al)	Ap	56	S1231

3.3 List and isolation of plant fungal pathogens used in this study

Two plant pathogenic fungi namely *F.spp* and *P. infestans* causing root rot/wilt in faba bean and late blight in potato, respectively, were used in this study.

Isolation of *Fusarium spp*.: Three soil samples (about 50 g) were collected by sterile plastic bags from sick plot of faba bean at APPRC. They were dried at room temperature, ground and sieved. One gram of each fine soil was suspended in 10 mL sterile water and the suspension was further serially diluted in sterile water. An aliquot of each soil suspension (0.5 ml) was then transferred to sterilized PDA plates using syringe. The inoculated plates were incubated at 26.4^{o}C for six days. On next day the growing organisms on the media were inspected and the suspected testing organism was sub-cultured twice using single hyphae isolation technique in replicate on a new PDA medium. Pure isolate was obtained and identified by professionals in APPRC based on its physical characteristics and comparison with previous cultures (Fig 34, 96-2A) [133].

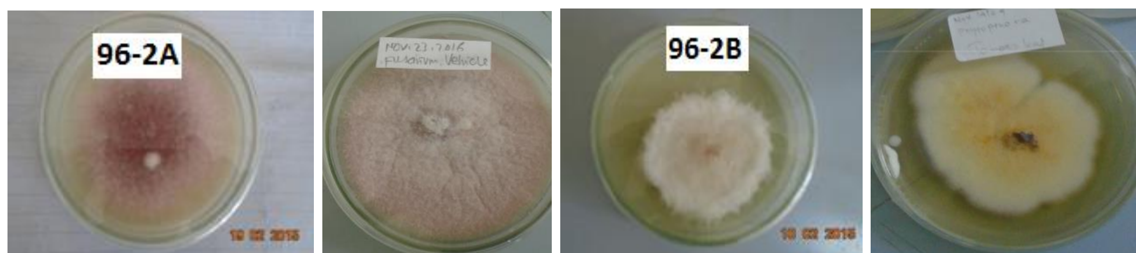


Fig. 34 Fungal isolates of faba bean (96-2A) and potato (96-2B) growing on PDA plate

Identified and preserved isolate of *P. infestans* was obtained from mycology lab (APPRC) and reinitiated to afford 96-2B. The two fungi were also isolated from diseased faba bean and potato leaves collected from field around Ambo using the same procedure above and found identical with their first isolate. These fungal isolates were maintained with periodic sub-culturing at APPRC in PDA.

3.4 *In vitro* antifungal activity assay (Food Poisoning)and statistical analysis

Plates with 7-14 day-old mycelium of the fungi preserved in APPRC were refreshed prior to the bioassay. The crude extracts, essential oils and compounds obtained in this study, and standard were dissolved in vehicle to the required concentration and subjected to *in vitro* test against growth of fungal pathogens under study following the modified method reported elsewhere [134, 135].

Media preparation: 41 g of PDA was added to distilled water (1 L) in a conical flask and magnetically stirred to dissolve while heating (30 min), autoclaved at 121°C (1 atm) for 15 minutes and cooled. The isolation room was first irradiated with UV for 30 min.

Food poisoning Bioassay method: One mL of each testing material (vehicle, 20 mg/mL extracts or fractions, 5 mg/mL of essential oils or compounds or standard) was pipetted to each petri dish in UV sterilized hood with laminar air flow. About 20 mL of the sterilized media was dispensed to each petri dish, agitated gently by hand for 1 min for a proper mixing of test material, and finally the poisoned media was allowed to solidify at room temperature in a hood for 24 h.

Inoculation and incubation: Next day about 6 mm diameter of mycelia containing agar disc was punched aseptically with a sterile needle/ cork borer from fungal culture, and the fungal slices were transferred using clean needle to the center of each petri dish

containing the test material. Petri dishes were sealed with para film and placed in an incubator at 26.4^{0}C for 2 weeks.

Data recording and analysis: All plates were prepared in three replicates arranged in completely randomized design (CRD) and each test was done twice. At two weeks the diameter of mycelial growth (G) per replicate petri dish was recorded in millimeter and the mean of the three replicates were calculated. The growth inhibition (GI) was found by subtracting G of the treatments from that of the control (vehicle) for all tests. The fungitoxicity effect of test materials in terms of percentage inhibition of mycelial growth (%GI) was manuplated by comparing the diameter of mycelia growth of treatments with control using the standard formula:

$$\%\ \text{Growth inhibition} = \frac{\text{Mycelial growth in control} - \text{Mycelial growth in treatment}}{\text{Mycelial growth in control}} X\ 100$$

The potential of plant materials to inhibit mycelial growth of fungi was categorized as high (%GI>65), moderate (65>%GI>50) and weakly active (inactive) (%GI<50) by taking the minimum %GI of the standard (68%) as bench mark. Relative Standard Deviation (%RSD) was calculated for all tests and inhibition zones obtained from the antifungal activity test were compared with control. Data was presented as mean ±%RSD.

3.5 Extraction and fractionation of plant extracts for antifungal and chemical study

Plant parts were sun dried, ground and placed in labeled bottles until used. The plant materials (20-100 g) were extracted using ethanol (95%) by maceration with shaking on shaker for 24 h. The extracts in solvent were concentrated under vacuum using rotary-evaporator to get solvent free crude extracts for bioassay or/and phytochemical study. The active plant extracts were further fractionated into two portions using hexane/ chloroform (1:1) and methanol, and subjected to antifungal assay and chemical study.

3.6 Isolation, purification and structure elucidations of compounds

General procedure: Separation of crude extract of a plant material into several fractions and purification to compounds was made by using combination of chromatographic techniques (CC, TLC, Sephadex LH-20) as shown in Fig. 35.

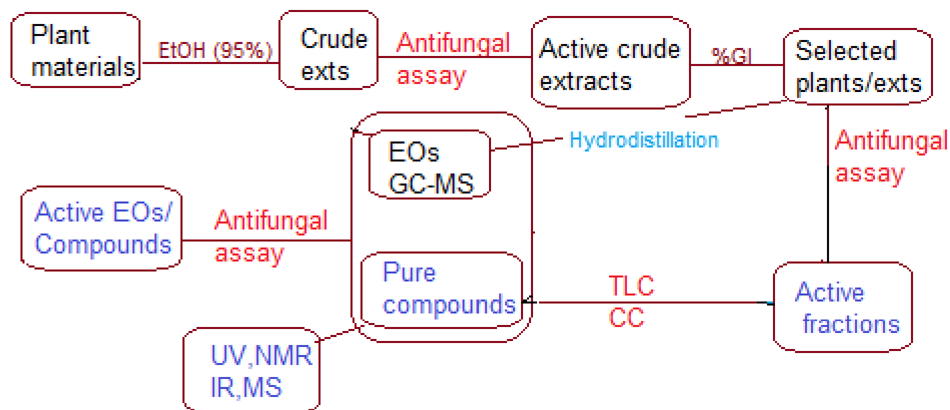


Fig. 35 Procedure for bioassay guided purifications of active principles

The structures of purified compounds were elucidated using the data generated by appropriate spectroscopic methods such as NMR, UV-VIS, IR, and MS. The ^{1}H-^{1}H correlations, one bond ^{1}H-^{13}C chemical shift correlations and long range ^{1}H-^{13}C bond correlations were also determined by using COSY, HSQC and HMBC experiments, respectively.

Isolations and semisynthesis of compounds and their characterizations: Specific isolation techniques, list of compounds isolated from each plant materials and their physical and spectroscopic data were presented. In addition derivatization procedures of dehydrocostus lactone and physical and spectroscopic data of the modified compounds were compiled.

***Inula confertiflora* leaf**: Four CC separations were made on leaf extracts specifically 96-1I, 96-24K, 96-29E, 96-38B and 96-47O. 96-II was partitioned into non polar (96-28E) and polar fractions (96-28F) using hexane: $CHCl_3$ (1:1) and methanol, respectively and submitted to antifungal assay. Nonpolar fraction of 96-29E was chromatographed on silica gel column (70-230 mesh) using hexane, ethyl acetate and methanol gradient and 80 fractions (40 mL) were collected. They were pooled to 21 fractions using their TLC profile. Graveolide (**131**) (96-31A, IC6A, 30 mg) and carabrone (**174**) (96-32G, IC6B, 65 mg) were isolated from Fr6 (96-30F) and Fr7 (96-30H) through recrystallizations in hex/EtOAc and Et_2O, respectively. β-sitosterol (**306**) (96-33I, IC5A, 10 mg) was isolated by purification of Fr 4 (96-30D) with $CHCl_3$/ MeOH. Similar CC fractionation of 96-38B gave three compounds namely *n*-hentriacontane (**476**) (96-40A, IC1B, 6 mg),

stigmasterol (**307**) (96-43A, IC5B,10 mg), carpesiolin (**139**) (96-45J, IC7,14 mg) through recrystallizations of Fr1(96-40A), Fr4 (96-40D), Fr7(96-40G) in hexane,EtOAc and EtOAc/Et_2O, respectively.

Graveolide: white crystal (30 mg) in Et_2O; mp 142-145^oC (Lit. [136] 152-154^oC); TLC: Rf 0.39 mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR v (cm^{-1}) 1769(-C=O, lactone), 1720 (C=O, ring ketone), 1656 (C=C), 2981/ 2930(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 1.94(1H, *m*, H-1), 1.63 and 2.10 (2H, *m*, H-2), 1.52(1H,m, H-3), 2.48(1H,*m*,H-3), 2.48 and 2.17 (2H,*m*,H-6), 2.81(1H,*qt, J*=15.2,12.4,9.6,3.6,3.2,H-7),4.28(1H,*ddd*, *J*=12.0,9.2,3.2,H-8), 1.42 and 2.48 (2H,*m*,H-9), 1.94(1H,*m*, H-10), 5.51(1H,*d*,*J*=3.2, H-13), 6.18(1H,*d*,*J*=3.6, H-13), 1.08 (3H,*d*,*J*=6, H-14), 1.04(3H,s,H-15); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 48.68(C-1),24.12(C-2),34.53(C-3), 221.19 (C-4), 50.01(C-5),35.19(C-6), 44.74(C-7), 80.83(C-8), 44.05 (C-9), 29.59 (C-10), 140.22 (C-11),169.86 (C-12),120.06 (C-13), 19.97 (C-14), 22.02 (C-15); LC-ESI-MS: m/z 249.14 $[M+H]^+$ (100%) for $C_{15}H_{21}O_3$.

Carabrone: colorless needles (37 mg) in Et_2O; mp 77-78^oC (Lit. [115] 90-91^oC); TLC: Rf 0.39, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR v (cm^{-1}) 1756(-C=O, lactone), 1706 (C=O, ring C=O), 1644 (C=C), 2979/ 2943(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 0.46(1H, *m*, H-1), 1.60 (2H, *m*, H-2), 2.53 (2H, *t,J*=7.2,H-3), 0.38 (1H,*m*,H-5), 0.95 and 2.35 (2H,*m*,H-6), 3.17(1H,*m*,H-7), 4.79 (1H,*ddd*,*J*=14.8,8.8,6.0,H-8), 0.95 and 2.35 (2H,*m*, H-9), 5.56 (1H,*d*,*J*=2.4, H-13), 6.25 (1H,*d*,*J*=2.8, H-13), 1.10 (3H,s, H-14), 2.17 (3H,s,H-15); ^{13}C NMR (100.5 MHz, $CDCl_3$); δ_C 34.23 (C-1),23.34 (C-2),43.59 (C-3), 208.74 (C-4), 22.90 (C-5), 30.74(C-6), 37.73 (C-7), 75.62 (C-8), 37.28 (C-9), 17.22 (C-10), 138.97 (C-11),170.51(C-12),122.62 (C-13), 18.24 (C-14), 30.12(C-15); EI-MS: m/z: 248.0 $[M^{+\cdot}]$ for $C_{15}H_{20}O_3$.

β-sitosterol: white solid (10 mg) in MeOH/ $CHCl_3$; mp 134-135^oC ; TLC: Rf 0.48, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no absorption; IR v (cm^{-1}) 3430 (-OH), 2930/2843 (C-H), 1693 (C=C); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 3.53 (1H, *m*, H-3), 5.37 (1H, *m*, H-6), 0.70 (3H,s,H-18), 1.03 (3H,s,H-19), 0.94 (3H,d, 6.4, H-21), 0.85 (9H,*m*, H-26,27,29); ^{13}C NMR (100.5 MHz, $CDCl_3$); δ_C37.25(C-1),31.67(C-2),71.82(C-3), 42.32 (C-4), 140.76 (C-5),121.73 (C-6),

31.67 (C-7), 31.91(C-8), 50.13 (C-9), 36.51(C-10), 21.09 (C-11), 39.78 (C-12),42.32 (C-13),56.77(C-14), 24.31(C-15), 28.25(C-16),56.06(C-17),11.86(C-18),19.40(C-19), 36.15 (C-20), 19.03 (C-21), 33.95 (C-22), 26.07(C-23), 45.84 (C-24), 29.15 (C-25),18.78 (C-26), 19.82(C-27),23.07(C-28), 11.99(C-29); MF$C_{29}H_{50}O$.

n-hentriacontane: white powder (6 mg) in hexane; mp 60-63°C (Lit. [111] 66-68°C); TLC: Rf 0.81, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no absorption; IR ѵ (cm^{-1}) 2923/ 2849 (C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 0.90 (6H,*t*,*J*=6.4,H-1,31),1.27 (58H, *br s*, H-2-30); ^{13}C NMR (100.5 MHz, $CDCl_3$); δ_C 14.13 (C-1,31), 22.71 (C-2,30), 31.95 (C-3,29), 29.38 (C-4,28), 29.72 (C-5-27). EI-MS m/z: 436.0 [$M^{+\cdot}$] for $C_{31}H_{64}$.

Stigmasterol: colorless solid (5 mg) in EtOAc; mp 145-147°C; TLC: Rf 0.48, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no absorption; IR ѵ (cm^{-1}) 3456 (-O-H), 2956/ 2918(=CH), 2856 (C-H), 1693 (C=C); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 3.55 (1H, *ddd*, *J*=16.0,11.2,4.8, H-3), 5.36 (1H, *d*,*J*=5.2, H-6), 0.71 (3H,s,H-18), 1.00 (3H,s,H-19), 0.82 (3H,d, 7.6, H-21), 5.17(1H,*dd*, *J*=15.2,8.4,H-22), 5.03(1H,*dd*, *J*=15.2,8.4,H-23), 0.82 (6H,*m*, H-27,29), 1.03(3H,*m*, H-26); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 37.26(C-1),31.67(C-2),71.82(C-3), 42.31 (C-4), 140.76(C-5),121.71(C-6), 29.70(C-7), 31.90(C-8), 50.17(C-9), 36.52(C-10), 21.07(C-11), 39.69(C-12), 42.22(C-13), 56.87(C-14) ,24.37(C-15), 28.91(C-16), 55.97(C-17), 12.25(C-18), 19.40(C-19), 40.49(C-20), 21.08 (C-21), 138.31(C-22), 129.28(C-23), 51.24 (C-24), 31.90(C-25), 21.22(C-26), 18.98(C-27), 25.41(C-28), 12.05(C-29); MF$C_{29}H_{48}O$.

Carpesiolin: white powder (37 mg) in Et_2O/EtOAc; mp 116-118°C (Lit. [121] 122-123 ^{o}C); TLC: Rf 0.33, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ѵ (cm^{-1}) 3406 (-O-H),1769(-C=O, lactone), 1744 (C=O, ring ketone), 1644 (C=C), 2981/ 2930/2869(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.23(1H, *m*, H-1), 2.23 and 1.51 (2H, *m*, H-2), 2.23 and 2.47 (2H,*m,* H-3), 4.04 (1H,*d*, *J*=8.8, H-6), 2.90 (1H,*m*,H-7), 4.40 (1H,*ddd*, *J*=12.0,10.0,2.8,H-8), 1.51 & 2.47 (2H,*m*,H-9), 1.85 (1H,*m*, H-10), 6.01(1H,*d*,*J*=3.2, H-13), 6.23(1H,*d*,*J*=3.6, H-13), 1.11(3H,*d*,*J*=6.8, H-14), 1.04 (3H,s,H-15), 1.70 (1H, br s); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 45.17 (C-1),24.60 (C-2),37.67 (C-3), 220.82 (C-4), 57.75 (C-5), 75.36 (C-6), 52.01 (C-7), 76.02 (C-8), 44.14 (C-9), 30.18 (C-10), 138.89 (C-

11),169.69 (C-12),121.95 (C-13), 20.09 (C-14), 19.09 (C-15); LC-ESIMS: m/z 265.14 $[M+H]^+$ (100%) for $C_{15}H_{21}O_4$

***Inula confertiflora* root:** It was extracted as usual three times and CC separations were made on crude extracts (96-33N, 96-38A, 96-48P). CC chromatography on 96-33N (4 g) using hexane and ethyl acetate of increasing polarity afforded nine pooled fractions. Fr5 (96-34E) was purified using hexane to afford stigmasterol (**307**) (96-35B, 4 mg). Fr6 was also purified by Et_2O and gave carabrone (**174**) (96-35C, 20 mg). Similarly 96-38A (3.5g) was applied on column and eluted with Hex, EtOAC and MeOH to afford seven pooled fractions. Epifriedelanol (**241**) (96-39H, 5 mg) was recrystalized from Fr2 (96-39B, 60 mg) in EtOAc. 96-48P (5 g) was also subjected to CC separation using hex, $CHCl_3$ and MeOH of increasing polarity and eleven pooled fractions were collected. Only Fr4 (96-48T, 30 mg) was found as colorless solid in eluting solvent and others were not successful. Decision was made to mix Fr 3-5 (96-38A) and Fr1-3 (96-48P) and it was rechromatographed in hex:EtOAc as mobile phase to afford fourteen fractions. Dammara-20, 24-dien-3-yl acetate (**245**) (96-56A, 25 mg) was recrystallized from Fr1 (96-51B) in EtOAc. Fr2 (96-51C) was found as oil with pleasant smell and its GC-MS analysis indicated the presence of thymol (**233**) at RT8.904. Stigmasterol (**307**) (96-51E or 59Q) from Fr4, graveolide (**131**) (96-51J) from Fr9 and carpesiolin (**139**) (96-51L) from Fr 11 were obtained.

Epifriedelanol: colorless solid (5 mg) in EtOAc; TLC: Rf 0.46, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no absorption; IR ν (cm^{-1}) 3490 (-O-H), 2925/ 2870(C-H); ^1H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 3.75 (1H, *d*, *J*=2.8, H-3), 0.90(3H, *d*,*J*=6.8, H-23), 0.87 (3H,s), 0.91 (3H,s), 0.94 (3H,s), 0.95(3H,s), 0.97(3H,s), 1.01(3H,*s*), 1.18(3H,*s*); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 15.78(C-1),35.16(C-2),72.76(C-3), 49.14(C-4), 37.08(C-5),41.70(C-6),17.54(C-7), 53.18 (C-8), 38.35 (C-9), 61.32 (C-10), 35.33(C-11), 30.63(C-12), 37.82(C-13), 39.66(C-14), 32.31(C-15), 36.06 (C-16), 30.02 (C-17), 42.79 (C-18), 35.53 (C-19), 28.18 (C-20), 32.79 (C-21), 39.27(C-22), 11.63 (C-23),16.40 (C-24), 18.24 (C-25), 18.66 (C-26), 20.12(C-27), 31.79 (C-28), 35.04(C-29), 32.08(C-30); EI-MS m/z: 428.0 $[M^{+\cdot}]$ for $C_{30}H_{52}O$.

Epifriedelanol and Thymol (96-48T): colorless solid (30 mg) in hex/EtOAc; TLC: Rf 0.46, mobile phase (EtOAc:Hex (1:1)); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz.

Epifriedelanol: ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 3.75 (1H, *d*, *J*=2.8, H-3), 0.90(3H, *d*,*J*=6.8, H-23), 0.87 (3H,s), 0.91 (3H,s), 0.94 (3H,s), 0.95(3H,s), 0.97(3H,s), 1.01(3H,*s*), 1.18(3H,*s*); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 15.79(C-1),35.15(C-2),72.80(C-3), 49.16(C-4), 37.10(C-5),41.71(C-6),17.55(C-7), 53.19 (C-8), 38.37 (C-9), 61.33 (C-10), 35.34(C-11), 30.64(C-12), 37.83(C-13), 39.67(C-14), 32.33(C-15), 36.08 (C-16), 30.02 (C-17), 42.81 (C-18), 35.55 (C-19), 28.18 (C-20), 32.81 (C-21), 39.28(C-22), 11.63 (C-23),16.40 (C-24), 18.25 (C-25), 18.66 (C-26), 20.12(C-27), 31.79 (C-28), 35.04(C-29), 32.09(C-30); MF$C_{30}H_{52}O$.

Thymol: ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 7.10(1H, *d*,J=7.6 H-3), 6.75 (1H, *d*,J=7.2, H-4), 6.60 (1H,*br s,* H-6), 3.18 (1H,m, H-7),1.26 (6H,d,J=0.8), 2.29(3H,s); ^{13}C NMR (100.5 MHz, $CDCl_3$); δ_C 152.57(C-1), 131.32 (C-2), 126.22 (C-3), 121.61(C-4), 136.58 (C-5), 116.01 (C-6), 26.71(C-7), 22.67(C-8,9), 20.87(C-10); MF$C_{10}H_{14}O$.

Dammara-20,24-dien-3-yl acetate: white crystal (25 mg) in EtOAc; mp149-150°C; TLC: Rf 0.44, mobile phase (EtOAc:Hex (1:1)); UV λ_{max} (EtOH) nm: no significant absorption; IR ν (cm^{-1}) 2963/ 2850(C-H), 1728(C=O); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 5.14 (1H,m,H-24), 4.72 and 4.74 (2H, *br s*, H-21), 4.50 (1H, *m* H-3), 1.27 (3H,s,H-18),1.33 (3H,s,H-19),1.63 (3H,s), 1.71(3H,s), 0.89 (3H,s), 0.87(3H,*s*), 0.99 (3H,*s*), 2.05 (3H,s); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 38.79 (C-1), 23.72 (C-2),80.93 (C-3), 37.91(C-4), 55.98 (C-5),18.18 (C-6), 35.36 (C-7), 40.49 (C-8), 50.88 (C-9),37.15(C-10), 21.39 (C-11), 24.94 (C-12), 45.30 (C-13), 49.43 (C-14), 31.38 (C-15), 27.08 (C-16), 47.81 (C-17), 16.28 (C-18), 15.65 (C-19), 152.72 (C-20), 107.48 (C-21), 34.16 (C-22), 28.92 (C-23),124.47 (C-24), 131.41 (C-25), 25.70 (C-26), 17.71(C-27), 27.98 (C-28), 15.90(C-29), 16.50 (C-30),170.98 (-C=O), 21.30 (Me); EI-MS m/z: 468.0 [$M^{+\cdot}$] for $C_{32}H_{52}O_2$.

***Inula confertiflora* flower**: It (100 g) was collected on Feb 13, 2017. It was extracted with EtOH (95%) to afford 10 g crude extract and the extract (96-56D) was chromatographed using hex:EtOAc as eluting solvent to collect 25 pooled fractions.

Colorless crystal (96-57M, 12 mg) in diethyl ether was obtained from Fr13 proved to contain both graveolide (**131**) and carabrone (**174**). Carpesiolin (**139**) (96-57S, 18 mg) was also isolated as white solid from Fr19. Further small CC purification of Fr 11(96-57K) using hex: chloroform: EtOAc gave stigmasterol (**307**) (96-59Q, 20 mg), inuviscolide (**107**) (96-59O, 15 mg) and 4, 5-epoxyinuviscolide (**122**) (96-59P, 10 mg).

Inuviscolide: gummy in $CHCl_3$;Hex (15 mg); TLC: Rf 0.25, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ν (cm^{-1}) 3448 (-O-H),1753(-C=O, lactone), 1660 (C=C), 2949(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.18(1H, *m*, H-1), 1.72 and 1.98 (2H, *m*, H-2), 1.71 & 1.83 (2H,*m,* H-3), 1.68 (1H,m, H-5), 1.21 and 2.31(1H,m,H-6), 2.67 (1H,*m*,H-7), 4.33 (1H,*m*,H-8), 3.20 and 2.56 (2H,*m*,H-9), 5.55(1H,*d*,*J*=3.2, H-13), 6.23(1H,*d*,*J*=3.6, H-13), 4.97(1H,*s,* H-14), 5.10 (1H,s,H-14), 1.22 (3H, s,H-15); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 46.93 (C-1), 26.34 (C-2),41.12 (C-3), 80.41 (C-4), 59.13 (C-5), 29.89 (C-6), 45.38 (C-7), 82.39(C-8), 40.72 (C-9), 146.63 (C-10), 139.60 (C-11),170.21 (C-12),120.46 (C-13), 111.71 (C-14), 24.11 (C-15); MF $C_{15}H_{20}O_3$.

4,5-epoxyinuviscolide: jelly in $CHCl_3$;Hex (10 mg); TLC: Rf 0.42, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: 275; IR ν (cm^{-1}) 1765(-C=O, lactone), 1656 (C=C), 2937(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.58(1H, *d*,J=8.8, H-1), 1.95 and 2.11 (2H, *m*, H-2), 1.75 and 1.86 (2H,*m,* H-3), 1.27 and 1.86 (2H,m, H-6), 2.99 (1H,m,H-7), 4.05 (1H,*ddd,J*=13.6,12.0,9.6,H-8), 2.30 and 1.86 (2H,*m*,H-9), 2.11 (1H,*m*,H-10), 5.51(1H,*d*,*J*=3.2, H-13), 6.22(1H,*d*,*J*=6, H-13), 0.95(3H,*d,J*=9.6, H-14), 1.38 (3H,s,H-15); ^{13}C NMR (100.5 MHz, $CDCl_3$);δ_C 47.73 (C-1), 30.58 (C-2),32.73 (C-3), 69.96 (C-4), 69.75 (C-5), 28.93 (C-6), 44.41 (C-7), 82.57(C-8), 40.42 (C-9), 34.60 (C-10), 139.07 (C-11),170.03 (C-12),119.84 (C-13), 14.64 (C-14), 15.54 (C-15); MF $C_{15}H_{20}O_3$.

***Echinops kebericho* root**: Its root was bought from AA on Dec 2014 and air dried. The powdered material (100 g) was extracted with ethanol (95%) to obtain 16 g extract (96-9K). The extract was partitioned with hexane+$CHCl_3$ (1:1) and methanol to afford 12 g nonpolar (96-28B) and 4 g polar fractions (96-28C), respectively. The nonpolar fraction was analyzed with TLC and showed similar components in hexane and chloroform fractions. Part of the nonpolar fraction (1 g) was column washed with hexane and the

solvent soluble portion gave white crystals. It was elucidated as dehydrocostus lactone (96-37D) (**108**).

Dehydrocostus lactone (DHCL): white needles in hexane (400 mg); mp 56-57°C; TLC: Rf 0.36, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ν (cm^{-1}) 3081(-C=CH$_2$), 1744 (-C=O), 1644 (C=C), 2956 (C-H); ^{1}H NMR (400 MHz,CDCl$_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.91(3H, m, H-1,5,7), 1.94 (2H, *m*, H-2), 2.52(2H,*m,* H-3), 3.94(1H,t,J=9.2 H-6), 2.20 and 1.42(1H,m,H-8), 2.20 and 2.52 (2H,m, H-9), 5.48(1H,*d*,*J*=3.2, H-13), 6.20(1H,*d*,*J*=3.6, H-13), 4.88 and 4.79 (2H,s, H-14), 5.04 (1H,d,J=1.6,,H-15), 5.24 (1H,d,J2.0,,H-15); ^{13}C NMR (100.5 MHz, CDCl$_3$);δ_C 47.55 (C-1), 30.29 (C-2),32.61 (C-3), 151.32(C-4), 45.08 (C-5), 85.28(C-6), 52.00 (C-7), 30.94(C-8), 36.31 (C-9), 149.24 (C-10), 139.72 (C-11),170.26 (C-12),120.20 (C-13), 112.58(C-14), 109.52 (C-15); EI-MS: m/z 230.96 [M$^{+\cdot}$] (100%) for $C_{15}H_{18}O_2$.

Modifications of DHCL

Alkaline hydrolysis: DHCL (275 mg) was dissolved in MeOH and 20% methanolic KOH (50 mL) was added and shaked. The mixture was refluxed for 8 h on water bath and cooled. Saturated NaCl was added and then after the product was extracted with chloroform (100 mL) and dried with anhydrous sodium sulphate. Small column Separation followed by removal of the solvent afforded black jelly material (175 mg, 56%) [137].

Compound 484: 96-56E: black gum; TLC: Rf 0.40, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ν (cm^{-1}) 1765(-C=O),2912(C-H); ^{1}H NMR (400 MHz,CDCl$_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.86(1H, m, H-1), 1.81 and 1.88 (2H, m, H-2), 2.45(2H, m, H-3), 2.79 (1H, m, H-5), 3.86 (1H, t,*J*=9.2 Hz, H-6), 2.39 (1H, m, H-7), 2.13 and 1.29 (2H, m, H-8), 2.00 and 2.41 (2H, m, H-9), 2.37 (1H, m, H-11), 3.61 (2H,m,H-13), 4.70 and 4.80 (2H, s, H-14), 5.12 and 4.99 (2H, d, J=1.6Hz, H-15),3.30 (3H,s,-OMe); ^{13}C NMR (CDCl$_3$, 100.5 (MHz): δ_C 46.91 (C-1), 30.15 (C-2), 32.57 (C-3), 151.79(C-4), 51.77 (C-5), 85.50 (C-6), 43.92 (C-7), 32.54 (C-8), 37.76 (C-9), 149.96 (C-10), 47.87 (C-11), 176.07 (C-12), 68.87(C-13), 111.66 (C-14), 108.96 (C-15), 59.20 (-OMe). EI-MS m/*z*: 262.0 [M$^{+\cdot}$] for $C_{16}H_{22}O_3$.

Reaction of DHCL with $NaBH_4$: To DHCL (500 mg) in MeOH (25 ml) $NaBH_4$ (1420 mg) was added and the mixture was stirred at 0^0C for 1 h. The solvent was removed and the residue was acidified with 0.1 N HCl. The solution was extracted with EtOAc to afford 350 mg of dried crude product and it was applied on column packed with silica gel using hex: $CHCl_3$ as eluent to afford milky oil (290 mg, 51%) [138].

Compound 484: 96-60B: yellow jel; TLC: Rf 0.40, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ѵ (cm^{-1}) 1788(-C=O), 2923(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 2.88 (1H, m), 1.82 (2H, m), 2.41 (2H, m), 2.81 (1H, m), 3.92 (1H, t,*J*=9.2 Hz), 2.39 (1H, m), 2.06 and 1.29 (2H, m), 2.37 and 1.92 (2H, m), 2.36 (1H, m), 3.62 (2H,m), 4.74 and 4.85 (2H, s), 5.18 and 5.02 (2H, d, J=1.6Hz), 3.33 (3H,s); ^{13}C NMR ($CDCl_3$, 100.5 (MHz): δ_C 46.99, 30.18, 32.58, 151.75, 51.81, 85.52, 44.04, 32.52, 37.75, 149.98, 47.95, 176.08, 69.00, 111.72, 109.06, 59.24.

Epoxidation of DHCL using m-CPBA. DHCL (490 mg) dissolved in 20 mL of CH_2Cl_2 was added to CH_2Cl_2 (20 mL) solution of meta-chloroperbenzoic acid (m-CPBA) (735 mg), and stirred in an ice bath for 4 h. The reaction mixture was washed, respectively, with 0.01 M NaOH (40 mL) and 40 mL of distilled water to get crude product (860 mg). Separation was made with small column chromatography using hex: EtOAc to afford a white gummy material (88 mg, 17%) [88].

Compound 430: 96-60O: white gum; TLC: Rf 0.34, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ѵ (cm^{-1}) 1763(-C=O), 2936(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 3.22 (1H, dd, *J*=8,8Hz, H-1), 1.92 and 2.23 (2H, m, H-2),1.71 and 2.23 (2H, m, H-3), 2.13 (1H, m, H-5), 4.06 (1H, m, H-6), 2.75 (1H, m, H-7), 2.23 and 1.46 (2H, m, H-8), 2.48 and 2.15 (2H, m, H-9), 6.18 and 5.48 (2H, d,J=3.2Hz, H-13), 4.95 and 4.99 (2H, s, H-14), 2.86 and 3.38 (2H, d,J=4.4Hz, H-15); ^{13}C NMR ($CDCl_3$, 100.5 (MHz): δ_C 46.91 (C-1), 28.54 (C-2), 31.53 (C-3), 66.46 (C-4), 53.36 (C-5), 81.95 (C-6), 46.30 (C-7), 29.66 (C-8), 33.32 (C-9), 148.25 (C-10), 139.53 (C-11), 169.76 (C-12), 120.29(C-13), 114.23 (C-14), 50.78 (C-15).

Michael addition of octylamine to DHCL**:** DHCL (230 mg) was dissolved in MeOH (5 mL) and octylamine (0.3 mL, $C_8H_{19}N$, ρ=0.782g/ml) was added to it. The mixture was stirred at room temperature for 24 h. After acidification using 0.1M HCl the reaction mixture was

supplemented with $CHCl_3$ and saturated sodium chloride, and the organic layer was separated and dried over anhydrous sodium sulphate to afford a solid (595 mg). It was applied on small column so as to get a yellow soilid (250 mg, 69.6%) [139].

Compound 485: 96-62B: yellow solid; mp 120-123°C; TLC: Rf 0.32, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ν (cm^{-1}) 3398 (N-H), 3086 (=CH_2), 1764(-C=O), 2966/2936(C-H); ^{1}H NMR (400 MHz,$CDCl_3$), chemical shift (δ), coupling constant *J* in Hz: δ_H 2.82(1H, m, H-1), 1.80 and 1.90 (2H, m, H-2), 2.46 (2H, m, H-3), 2.82 (1H, m, H-5), 3.98 (1H, t,*J*=9.2 Hz, H-6), 2.26 (1H, m, H-7), 2.15 and 1.38 (2H, m, H-8), 2.46 and 2.05 (2H, m, H-9), 2.82 (1H, m, H-11), 3.05 (2H,d, *J*=6Hz, H-13), 4.78 and 4.85 (2H, s, H-14), 5.18 and 4.99 (2H,s,H-15), 2.82 (2H,m,H-1'), 1.65 (2H,m,H-2'), 1.23(10H,m,H-3',4',5',6',7'), 0.85 (3H,t, *J*= 6.8Hz, H-8'), 6.48 (1H,br.s); ^{13}C NMR ($CDCl_3$, 100.5 (MHz): δ_C 46.88 (C-1), 30.08 (C-2), 32.49 (C-3), 151.66(C-4), 51.76 (C-5), 86.28 (C-6), 45.40 (C-7), 32.35 (C-8), 37.51 (C-9), 149.58 (C-10), 45.50 (C-11), 177.56 (C-12), 46.95 (C-13), 111.93 (C-14), 109.01 (C-15), 49.53 (C-1'), 27.92 (C-2'), 27.01(C-3'),29.29(C-4'),29.18(C-5'),31.74(C-6'), 22.60 (C-7'),14.06 (C-8'). EI-MS m/z: 359.0 [$M^{+\cdot}$] for $C_{23}H_{37}NO_2$.

Reaction of DHCL with Iodine/ Benzene: To 250 mg solution of DHCL in benzene (10 ml) catalytic iodine (20 mg) was added and refluxed for 12 h under nitrogen atmosphere at 81^{0}C. The reaction mixture was diluted with water (10 mL) and extracted with diethyl ether. The last traces of iodine from the organic layer was removed by washing thoroughly with sodium thiosulphate and finally dried over sodium sulphate. Evaporating the solvent resulted in thick brown oil (230 mg, 92%) [89].

Catalytic Hydrogenation of DHCL (Attempted): Two balloons were filled with hydrogen gas manually from gas cylinder. DHCL (230 mg) in MeOH (5 mL) and 5%Pd/C (23 mg) were added to two necked round bottom flask and the mixture was stirred at room temperature (ca. 20°C). The air inside the flask was replaced with hydrogen gas from the balloon attached at one neck and then after the other hydrogen filled balloon was attached to the open neck. The reaction system was stirred under hydrogen gas for 6 h. The reaction mixture was filtered using filter paper and concentrated. It was applied on PTLC to get colorless oily product (35 mg) [140]. The NMR data of the sample showed

reductions of all double bonds and the number of carbon was more than expected and unpredictable.

Artemisia annua: Leaf of the plant was collected from ALNAP garden on June 2015 and dried. Ground leaf (100g) was extracted with ethanol (95%) as usual to afford 15 g extract. It was fractionated into nonpolar (96-50A, 10 g) and polar parts (5 g). About 8 g of 96-50A was applied on column packed with silica gel (70-230 mesh) and eluted with pet.ether: EtOAc: MeOH of increasing polarity to afford 20 fractions. Three compounds namely 1-heptacosanol (**486**) (96-50D)**,** artemisinin (**445**) (96-50J) and scopoletin (**404**) (96-50P) were isolated through small column fractionation and recrystallizations from Fr 5,8 and 14 respectively. In large scale CC separation of the same material (96-56C, 15 g) artemisinic acid (**447**) and dihydroartemisinic acid (**448**) were obtained as a mixture (96-60D) from Fr15 after further column filtration. The structures of the compounds were elucidated using the physical and spectroscopic data generated and literature.

1-Heptacosanol: white powder in Et_2O (30 mg); mp 73-75^oC; TLC: Rf 0.50, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no absorption; IR ν (cm^{-1}) 3336 (O-H), 2923/ 2837(C-H); ^1H NMR (400 MHz,$CDCl_3$), chemical shift (δ), coupling constant *J* in Hz: δ_H 3.66(2H, *t*,*J*=6.4 H-1), 1.58 (2H, *m*, H-2), 1.30 (48H,*br s,* H-3-26), 0.90(3H,m*, t*,*J*=6.7, H-27); ^{13}C NMR (100.5 MHz, $CDCl_3$); δ_C 63.12(C-1), 32.81(C-2),31.94(C-3),30.03-29.39(C-4-24),25.75(C-25), 22.72(C-26),14.15(C-27); MF$C_{27}H_{56}O$.

Artemisinin: white crystal (200 mg); mp 141-142^oC; TLC: Rf 0.48, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: no significant absorption; IR ν (cm^{-1}) 1739 (-C=O), 2974/ 2849 (C-H); ^1H NMR (400 MHz,$CDCl_3$), chemical shift (δ) in ppm, coupling constant *J* in Hz: δ_H 1.40 (1H, m, H-1), 2.05 and 1.48 (2H, m, H-2),2.43 and 2.08 (2H, m, H-3), 5.89 (1H, s, H-5), 1.78 (1H, m, H-7), 1.90 and1.07 (2H, m, H-8), 1.07 and 1.78 (2H, m, H-9), 1.43 (1H, m, H-10), 3.40 (1H,m,H-11), 1.23 (3H, d,J=7.2, H-13), 1.00 (3H, d,J=6.4, H-14), 1.45 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100.5 (MHz): δ_C 50.04 (C-1), 24.85 (C-2), 35.89 (C-3), 105.39 (C-4), 93.72 (C-5), 79.51(C-6), 44.95(C-7), 23.40 (C-8), 33.59(C-9), 37.52(C-10), 32.89(C-11), 172.11(C-12), 12.57(C-13), 19.84 (C-14), 25.21(C-15).MF $C_{15}H_{22}O_5$

Scopoletin: yellow crystal in DCM (20 mg); mp 198-200^oC; TLC: Rf 0.41, mobile phase (EtOAc:Hex (1:1)); UV λ_{max}(EtOH) nm: 295,340; IR ν (cm^{-1}) 3338 (O-H),1702(-C=O),

1614/1564(benzene); ^{1}H NMR (400 MHz, DMSO-d6), chemical shift (δ), coupling constant *J* in Hz: δ_H 6.21(1H, *d*,J=9.6H-2), 7.91 (1H, *d*, J=9.6, H-3), 7.21(1H, *s,* H-5), 6.78 (1H,s, H-8), 3.81(3H,s,OMe); ^{13}C NMR (100.5 MHz, DMSO-d6); δ_C 161.15(C-1), 110.04(C-2),144.95(C-3),110.95(C-4),112.08(C-5),145.74(C-6),151.69(C-7),103.21(C-8),149.98 (C-9), 56.46(C-10); MF$C_{10}H_8O_4$.

Artemisinic acid and dihydroartemisinic acid: colorless oil (8 mg); TLC: Rf 0.49, mobile phase (EtOAc:Hex (1:1)); IR ѵ (cm^{-1}) 3500 (shoulder) (-O-H),1714(-C=O), 2911(C-H); Artemisinic acid**:** ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ_C 41.37 (C-1), 25.55 (C-2), 26.38 (C-3), 134.99 (C-4), 120.16 (C-5), 37.88(C-6), 42.03(C-7), 25.94 (C-8), 35.23(C-9), 27.56(C-10), 142.60(C-11), 172.27(C-12), 126.55(C-13), 19.73 (C-14), 23.68(C-15). Dihydroartemisinic acid: ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ_C 43.60 (C-1), 33.98 (C-2), 27.40 (C-3), 136.00 (C-4), 119.30 (C-5), 42.14(C-6), 41.73(C-7), 26.61 (C-8), 25.76(C-9), 27.66(C-10), 36.35(C-11), 183.29(C-12), 15.07(C-13), 19.68 (C-14), 23.80(C-15).

3.7 Isolation, analysis and identification of components of Essential oils

Essential oils were isolated from *E. globulus* (leaf), *T. schimperi* (leaf), *I. confertiflora* (root, leaf, flower) and *A. annua* (leaf) by hydrodistilation of the plant materials (50 g) using Clevenger type apparatus for 2 h. The aqueous distillate was fractioned with chloroform into the organic and aqueous phases using separatory funnel. As soon as the organic phase was separated it was dried with anhydrous sodium sulfate and solvent was removed using rotary evaporator. The essential oil was weighed and preserved in a sealed vial at 4°C until the moment of bioassay and /or GC-MS analysis.

Essential oil componenets were separated and identified on GC-MSD system (Agilent technologies 7820A) coupled to a mass detector (5977E MSD) and HP-88 column (30 m x 0.25 mm x 0.25 µm film thickness) coated with 100% poly (dimethylsiloxane). Samples (1 µL) were injected and helium gas was used as carrier gas. The total run time was set to 31.31 minutes. The identification of the constituents was based on a comparison of their retention indices relative to those of the literature. Further identification was made by matching their recorded mass spectra with those stored in the mass spectral library (NIST) of the GC–MSD data system.

4. Conclusion and Recommendations

In this study, ethanol crude extracts from *I. confertiflora, E. kebericho* and *A. annua, and* essential oils obtained from *E. globulus* and *T. schimperi* were found more active and considered as prospective sources of antifungal compounds. Bioassay guided phytochemical investigations on nonpolar fractions of *I. confertiflora, E. kebericho* and *A. annua* afforded fourteen compounds and their structures were elucidated as inuviscolide (**107**), 4, 5-epoxyinuviscolide (**122**), graveolide (**131**), carpesiolin (**139**), carabrone (**174**), epifriedelanol (**241**), dammara-20,24-dien-3-yl acetate (**245**), β-sitosterol (**306)**, stigmasterol (**307**), *n*-hentriacontane (**476)**, dehydrocostus lactone (DHCL) (**108**), scopoletin (**404**), artemisinin (**445**) and 1-heptacosanol (**486)**. *I. confertiflora* was chemically investigated for the first time and in the course ten compounds were isolated.

The GC-MS analysis of the essential oil isolated from *I. confertiflora* root showed 2,5-dimethoxy-4-isopropyltoluene (32%)(**479**) and thymol (31%) (**233**) as dominant components while the flowers oil was composed of mainly **479** (25%) and thymylisobutyrate (16%) (**234**). Camphor (81%) (**443**) was identified as the major component of the essential oil obtained from *A. annua* leaves. Eucalyptol (90%) (**397**) and **233** (72%) were found the major constituents of leaf essential oils extracted from *E. globulus* and *T. schimperi,* respectively.

The *in vitro* antifungal activity study of isolated compounds confirmed that **108**, **131**, **139**, **174** and **445** were more potent, of which **108** and **174** found promising antifungal compounds for the control of the fungi under study. Analoques of DHCL were synthesized and their growth inhibition potential demonstrated weaker antifungal activity which implies the role of α-methylene-γ-lactone unit in determining the antifungal activity of DHCL. Two of the synthesized compound structures were not yet reported.

The observed laboratory scale inhibitory activity of potent extracts need further evaluation in green house and field experiments. The result of this *in vitro* antifungal activity study is also an important indicator for the possibilities of using natural plant products as biofungicides in the control of plant diseases caused by *F. spp.* in faba bean and *P. infestans* in potato.

5. References

1. Observatory of Economic Complexity: OEC: Ethiopia (http://atlas.media.mit.edu/profile/country/eth/ accessed on 06 June, 2017)
2. Central Statistic Authority (CSA). **2016**. Agricultural sample survey 2015/ 2016 (2008 E.C.). Volume I Report on area and production of major crops (private peasant holdings, Meher season) May 2016. Statistical bulletin, 584.pp1-121 (http://www.csa.gov.et/, accessed on 06 June, 2017).
3. Abraham, T. (ed.). **2008**. Increasing Crop Production through Improved Plant Protection-Volume I. Proceedings of the 14th Annual Conference of the Plant Protection Society of Ethiopia (PPSE), 19-22 December 2006, Addis Ababa, Ethiopia. pp598.
4. Dereje, G. (ed). **2013**. Seed Potato Tuber Production and Dissemination Experiences, Challenges and Prospects. Proceedings of the National Workshop on Seed Potato Tuber Production and Dissemination, 12-14 March 2012, Bahir Dar, Ethiopia, EIAR and ARARI, 2013,pp338.
5. Navarre, D.A.; Goyer, A.; Shakya, R. **2009**. Nutritional Value of Potatoes: Vitamin, Phytonutrient, and Mineral Content USDA-ARS, USA. (http://naldc.nal.usda.gov/download/37634/PDF accessed on Sep 2014).
6. Abraham T. (ed.). **2009**. Increasing Crop Production through Improved Plant Protection-Volume II. Proceedings of the 14th Annual Conference of the Plant Protection Society of Ethiopia (PPSE), 19-22 December 2006, Addis Ababa, Ethiopia. pp542.
7. Habtamu, K.; Alemayehu, C.; Bekele, K.; Pananjay, G.B.G.; Tiwari, K. **2012**. Evaluation of different potato varieties and fungicide combinations for the management of potato late blight (*Phytophthora infestans*) in Southern Ethiopia. *International Journal of Life Sciences* **1**, 8-15.
8. Bekele, K. **2004**. Ethiopia late blight profile. Ethiopian Agricultural Research Organization, Holetta Agricultural Research Center-Progress Report, Addis Ababa, Ethiopia.
9. Akibode, S.; Maredia, M. **2011**. Global and Regional Trends in Production, Trade and Consumption of Food Legume Crops, Department of Agricultural,

Food and Resource Economics, Michigan State University Report Submitted to SPIA.(http://impact.cgiar.org/sites/default/files/images/Legumetrendsv2.pdf.accessed on Sep.2014)

10. Agrios, G. N. **2005**. Plant Pathology. 5th ed. Elsevier Academic Press, Burlington, Mass., USA.
11. Teklay, A.; Tsehaye, B.; Yemane, N.; Assefa, W. **2014**. The Prevalence and Importance of Faba Bean Diseases with Special Consideration to the Newly Emerging “Faba Bean Gall” in Tigray, Ethiopia. *Discourse Journal of Agriculture and Food Sciences* **2**, 33-38.
12. Abdel-Kader, M.M.; El-Bahr, M.K.; El-Mougy, N. S. **2004**. Pathogenic Fungi and Soil Conditions Causing Root Rot and Wilt Disease Complex during Acclimatization of Tissue Culture-Derived Banana Plantlets. *Egyptian Journal of Phytopathology* **32**, 37-48.
13. Abdel-Monaim, M. F. **2013**. Improvement of Biocontrol of Damping-off and Root Rot/Wilt of Faba Bean by Salicylic Acid and Hydrogen Peroxide. *Mycobiology* **41**, 47-55.
14. Infantino, A.; Kharrat, M.; Riccioni, L.; Coyne, C. J.; McPhee, K. E.; Gr¨unwald, N. J. **2006**. Screening techniques and sources of resistance to root diseases in cool season food legumes. *Euphytica* **147**, 201–221.
15. Amin, M.; Mulugeta, N.; Selvaraj, T. **2013**. Field Evaluation of New Fungicide, Victory 72 WP for Management of Potato and Tomato Late Blight (Phytophthora infestans (Mont) de Bary) in West Shewa Highland, Oromia, Ethiopia. *Journal of Plant Pathology and Microbiology* **4**, 192-195.
16. Disease management: cultural management practices http://bugs.bio.usyd.edu.au/learning/resources/PlantPathology/disease_mgmt/cultural_mgmt.html (accessed on June 2017)
17. Farfour, S. A; Al-Saman; Mahmoud, A. **2014**. Root-rot and stem-canker control in Faba bean Plants by using some biofertilizers agents. *Journal of Plant Pathology and Microbiology* **5**, 1-4.

18. Abdulwahid, O.A. **2006**. Improving control of Fusarium wilt of leguminous plants by combined application of biocontrol agents. *Phytopathologia Mediterranea* **45**, 231–237.
19. Meki, S.; Ahmed, S.; Sakhuja, P.K. **2011**. Control of chickpea wilt (Fusarium oxysporum f.sp. ciceris) using *Trichoderma* spp. in Ethiopia. *Archives of Phytopathology and Plant Protection* **44**, 432–440.
20. Guest, D.; Brow, J. Plant defenses against pathogens (www.appsnet.org/.../17%20Defence%20mechanisms%20(DIG%26JFB))
21. Guleria, S.; Tiku, A.K. **2009**. Botanicals in Pest Management: Current Status and Future Perspectives (Chapter 12), pp317-329. (http://link.springer.com/chapter/10.1007%252F978-1-4020-8992-3_12)
22. Sultana, N.; Ghaffar, A. **2010**. Effect of Fungicides, Microbial antagonists and Oilcakes in the control of *Fusarium solani,* The Cause of seed rot, seedling and root infection of Bottle gourd, Bitter gourd and Cucumber. *Pakistan Journal of Botany* **42**, 2921-2934.
23. Muzubuti, E.S.G. **2007**. Management of Late blight with alternative products. *Pest Technology* **I**, 106-116.
24. Soytong, K.; Ratanacherdchai, K. **2005**. Application of mycofungicide to control late blight of potato. *Journal of Agricultural Technology* **1**, 19-32.
25. Dang, Q.L.; Lim, C.H.; Kim, J-C. **2012**. Current status of botanical pesticides for crop protection. *Research in plant Diseases* **18**, 175−185.
26. Dayan, F. E.; Cantrell, C. L.; Duke, S. O. **2009**. Natural products in crop protection. *Bioorganic and Medicinal Chemistry* **17**, 4022–4034.
27. Gurjar, M. S.; Ali, S., Akhtar; M., Singh, K.S. **2012**. Efficacy of plant extracts in plant disease management. *Agricultural Sciences* **3**, 425-433.
28. Debashri, M.; Tama, M. **2012**. A Review on efficacy of *Azadirachta indica* A. Juss based biopesticides: An Indian perspective. *Research Journal of Recent Sciences* **1,** 94-99.
29. Maharjan, B.L.; Shrestha, K.; Basnyat, S. **2010**. Botanical Control of Late Blight of Potato. *Nepal Journal of Science and Technology* **11**, 37-40.

30. Goufo, P.; Teugwa, C.M.; Fontem, D. A.; Ngnokam, D. **2008**. High efficacy of extracts of Cameroon plants against tomato late blight disease. *Agronomical Sustainable Development* **28**, 567–573.
31. Tadesse, M.; Daniel, N. **2005**. Potential of extracts of some Ethiopian medicinal plants for late blight control in organic potatoes. Poster at: Researching Sustainable Systems. International Scientific Conference on Organic Agriculture, Adelaide, Australia, September 21-23.
32. Zoubiri, S.; Baaliouamer, A. **2012**. Chemical composition and insecticidal properties of *Lantana camara*L. Leaf essential oils from Algeria. *The Journal of Essential Oil Research* **24**, 377–383.
33. Srivastava, D.; Singh, P. **2011**. Antifungal Potential of Two Common Weeds against Plant Pathogenic Fungi spps. Alternaria. *Asian Journal of Experimental Biological Science* **2**, 525-528.
34. Saha, S.; Walia, S.; Kundu, A.; Kumar, B.; Joshi, D. **2012**. Antifungal Acetylinic Thiophenes from *Tagetes minuta*: Potential Biopesticide. *Journal of Applied Botany and Food Quality* **85**, 207-211.
35. Sadia, S.; Khalid, S.; Qureshi, R.; Bajwa, A.A. **2013**. *Tagetes minuta* L., A useful underutilized plant of family *Asteraceae*: A review. *Pakistan Journal of Weed Science Research* **19**, 179-189.
36. Isman, M. B. **2008**. Perspective Botanical Insecticides: for richer, for poorer. *Pest Management Science* **64**, 8–11.
37. ENY-350 (IN197). **2009**. A series of the Department of Entomology and Nematology, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. (http://www.edis.ifas.ufl.edu.)
38. Bowers, J. H.; Locke, J. C. **2000**. Effect of botanical extracts on the population density of Fusarium *oxysporum* in soil and control of Fusarium wilt in the greenhouse. *Plant Disease* **84**,300-305.
39. Duke, S. O.; Cantrell, C. L.; Meepagala, K. M.; Wedge, D. E.; Tabanca,N.; Schrader, K. K. **2010**. Review: Natural Toxins for Use in Pest Management. *Toxins* **2**, 1943-1962.

40. Isman, M. B. **2006**. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* **51**, 45–66.
41. Salatino, A.; Faria Salatino, M. L.; Negri, G. **2007**. Review. Traditional uses, Chemistry and Pharmacology of Croton species (Euphorbiaceae). *Brazilian Chemical Society* **18**, 11-33.
42. Okemo, P.O. ; Bais, H.P. ; Vivanco, J. M. **2003**. *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens. *Fitoterapia* **74**, 312–316.
43. Zoubiri, S.; Baaliouamer, A. **2012**. Chemical composition and insecticidal properties of *Lantana camara* L. leaf essential oils from Algeria. *The Journal of Essential Oil Research* **24**, 377–383.
44. Chandra, R.D.; Munan, S. M.D. **2013**. Toxicology, Phytochemistry, Bioactive Compounds and Pharmacology of *Parthenium hysterophorus*. *Journal of Medicinal Plants Studies* **1**, 126-141.
45. Shafique, S. **2012**. Biological Control Potential of *Parthenium hysterophorus* against *Fusarium solani*–A Cause of Fusarium Wilt in Potato. International Conference on Applied Life Sciences (ICALS2012) Turkey, September 10-12, pp315-320.
46. Dissanayake, M.L.M.C.; Jayasinghe, J. **2013**. Antifungal activity of selected medicinal plant extracts against plant pathogenic fungi; *Rhizoctonia solani, Colletotrichum musea and Fusarium oxysporum. International Journal of Science Inventions Today* **2**, 421-431.
47. Chapagain, B.P.; Wiesman, Z.; Tsror, L (Lahkim). **2007**. In vitro study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. *Industrial Crops and Products* **26**, 109–115.
48. Prabhu, K.S.; Lobo, R.; Shirwaikar, A.A.; Shirwaikar. **2009**. *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. *The Open Complementary Medicine Journal* **1**, 1-15.
49. Zia-ul-haq; Ahmad, M.; Mansoor, M.; Jehan, N.; Ahmad, S.; Qayum, M.; Marwat, I. K. **2011**. Antimicrobial Screening of Selected Flora of Pakistan. *Archeve of Biologcal Science* **63**, 691-695.

50. Pawar, V.C; Thaker, V.S. **2007**. Evaluation of the anti-*Fusarium oxysporum* f. sp *cicer* and anti-*Alternaria porri* effects of some essential oils. *World Journal of Microbiology and Biotechnology* **23**, 1099–1106.
51. Prajna, J.; Richa, J.; Dipjyoti, C. **2013**. HPLC Quantification of Phenolic Acids from *Vetiveria zizanioides* (L.) Nash and Its Antioxidant and Antimicrobial Activity. *Journal of Pharmaceutics*, 1-6.
52. Shehab, G.M.G.; Ahmed, O.K.; Ahmad, M.A. **2013**. *Jatropha curcas* (Linn) Seed Ethanolic Extract Possess Antifungal Activity against *Phytophthora infestans* and Stimulates Potato Resistant Response. *Journal of Biological and Chemical Research* **30,** 929-941.
53. Kumar, A.; Sharma, S. **2008**. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. *Industrial crops and products* **1**, 1-10.
54. Seca, A. M.L.; Grigore, A.; Pinto, D. C.G.A.; Silva, A. M. **2014**. Review: The genus *Inula* and their metabolites: From ethnopharmacological to medicinal uses. *Journal of Ethnopharmacology* **154**, 286–310.
55. Zhao, Y-M; Zhanga, M-L; Shi, Q-W; Kiyota, H. **2006**. Chemical Constituents of Plants from the Genus *Inula*. *Chemistry and biodiversity* **3**, 371-384.
56. Seca, A. M.; Pinto, D.C.; Silva, A. M. **2015**. Metabolomic Profile of the Genus *Inula*. *Chemistry and biodiversity* **12**, 859-906.
57. Bessada, S. M. F.; Barreira, J. C. M.; Oliveira, M. B. P. **2015**. Asteraceae species with most prominent bioactivity and their potential applications: A review. *Industrial Crops and Products* **76**, 604–615.
58. Gebre-Mariam, T.; Neubert, R.; Schmidt, P.C.; Wutzler, P.; Schmidtke, M. **2006**. Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders. *Journal of Ethnopharmacology* **104**,182–187.
59. Mesfin, T. **2004**. Asteraceae (Compositae): Flora of Ethiopia and Eritrea, **4** (2), 122.

60. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. **2013**. Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecular Science* **14**, 12780-12805.
61. Omezzine, F.; Daami-Remadi, M.; Rinez, A.; Ladhari, A.; Haouala, R. **2011**. *In vitro* assessment of Inula spp. organic extracts for their antifungal activity against some pathogenic and antagonistic fungi. *African Journal of Microbiology Research* **5**, 3527-3531.
62. Wang, W.; Ben-Daniel, B. H.; Cohen, Y. **2004**. Control of Plant Diseases by Extracts of *Inula viscosa*. *Disease Control and Pest Management* **94**, 1042-1047.
63. Gökbulut, A.; Sarer, E. **2013**. Isolation and quantification of alantolactone/ isoalantolactone from the roots of *Inula helenium subsp. Turcoracemosa. Turky Journal of Pharmaceutical Science* **10**, 447-452.
64. Yan, H.; Haiming, S.; Cheng, G.; Xiaobo, L. **2012**. Chemical constituents of the roots of *Inula helenium*. *Chemistry of Natural Compounds* **48**, 522-524.
65. Burdi, D.R.; Hasan, M.; Uddin, V. **1991**. Sterols and a glycoside from the flowers of *Inula grantioides*. *Pakistan Journal of Pharmaceutical Sciences* **4**, 131-136.
66. Andolfi, A.; Zermane, N.; Cimmino, A.; Avolio, F.; Boari, A.; Vurro, M.; Evidente, A. **2013**. Inuloxins A–D, phytotoxic bi-and tri-cyclic sesquiterpene lactones produced by *Inula viscosa*: Potential for broomrapes and field dodder management. *Phytochemistry* **86**,112–120.
67. Bourrel, C.; Vilarem, G.; Perineau, F. **1993**. Chemical Analysis, Bacteriostatic and Fungistatic Properties of the Essential Oil of Elecampane (*Inula helenium* L.). *Journal of Essential Oil Research* **5**, 411-417.
68. Haoui, E.; Derriche, R.; Madani, L.; Oukali, Z. **2015**. Analysis of the chemical composition of essential oil from Algerian *Inula viscosa* (L) Aiton.Imid. *Arabian Journal of Chemistry* **8**, 587–590.
69. Kiliç, Ö, **2014**. Chemical Composition of Two *Inula sp.* (Asteraceae) Species from Turkey. *Igdir University Journal of the Institute of Science and Technology* **4**, 15-19.

70. Mesfin, T. **2004**. Asteraceae (Compositae): Flora of Ethiopia and Eritrea **4** (2), 21.

71. Fokialakis, N.; Osbrink, W.L.A.; Mamonov,L. K.; Gemejieva, N. G.; Mims, A. B.; Skaltsounis, A. L.; Lax, A. R.; Cantrell, C. L. **2006**. Antifeedant and toxicity effects of thiophenes from four Echinops species against the Formosan subterranean termite, Coptotermes formosanus. *Pest Management Science* **62**, 832–838.

72. Fokialakis, N.; Cantrell, C. L.; Duke, S.O.; Skaltsounis, A. L.; Wedge, D. E. **2006**. Antifungal Activity of Thiophenes from *Echinops ritro*. *Journal of Agriculture and Food Chemistry* **54**, 1651−1655.

73. Zhang, P.; Liang, D.; Jin, W.; Qu, H.; Cheng, Y.; Li, X.; Ma, Z. **2009**. Cytotoxic Thiophenes from the Root of *Echinops grijisii* Hance. *Z. Naturforsch* **64**c, 193–196.

74. Yinebeb, T.; Ariaya, H.; Asrat, H.; Rohloff, J. **2011**. *In vitro* Evaluation of Antileishmanial Activity and Toxicity of Essential Oils of *Artemisia absinthium* and *Echinops kebericho*. *Chemistry and biodiversity* **8**, 614-623.

75. Senejoux, F.; Demougeot, C.; Karimov, U.; Muyard, F.; Kerramb, P.; Aisa, H. A.; Girard-Thernier, C. **2013**. Chemical constituents from *Echinops integrifolius*. *Biochemical Systematics and Ecology* **47**, 42–44.

76. Frolova, V.I.; Ban'kovskii, A. I.; Zheleznova, E. S. **1957**. Chemical study of the alkaloids of *Echinops ritro. Medicinal Promst. SSSR* **11**, 20-24.

77. Chevrier, M. **1976**. The flavonoids of *Echinops ritro*. *Fitoterapia* **47**,115-117.

78. Khadim, E.J.; Abdulrasool, A.A.; Awad, Z.J. **2014**. Phytochemical Investigation of Alkaloids in the Iraqi *Echinops heterophyllus* (Compositae). *Iraqi Journal of Pharmaceutical Science* **23**, 26-34.

79. Berhanu, M. A.; Mesfin, T.; Majindm, R. **1991**. Distribution of Sesquiterpene Lactones and Polyacetylenic Thiophenes in Echinops. *Biochemical systematics and ecology* **19**,323-328.

80. Di Cosmo, F.; Towers, G.H.N; Lam, J. **1982**. Photoinduced fungicidal activity elicited by naturally occurring thiophene derivatives. *Pesticide Science* **13**, 589–594.

81. George, J.; Bais, H. P.; Ravishankar, G.A. **2000**. Biotechnological production of plant based insecticides. *Critical Review of Biotechnology* **20**, 49–77.
82. Madhuri, K.; Elango, K.; Ponnusankar, S. **2012**. *Saussurea lappa* (Kuth root): Review of its traditional uses, phytochemistry and pharmacology. *Oriental Pharmacy and Experimental Medicine* **12**, 1–9.
83. Chaturvedi, D. **2011**. Sesquiterpene lactones: Structural diversity and their biological activities. *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry* **10**, 313-334.
84. Rigby, J. H.; Wilson, J. Z. **1984**. Total synthesis of Guaianolides: (±)-Dehydrocostus lactone and (±)-Estafiatin. *Journal of American Chemical Society* **106**, 8217-8224.
85. Jia, Q-Q.; Wang, J-C.; Long, J.; Zhao, Y.; Chen, Si-J.; Zhai, J-D.; Wei, L-B.; Zhang, Q.; Chen, Y.; Long, H-B. **2013**. Sesquiterpene Lactones and Their Derivatives Inhibit High Glucose-Induced NF-κB Activation and MCP-1 and TGF-β1 Expression in Rat Mesangial Cells. *Molecules* **18**, 13061-13077.
86. Setia, R. R.; Kaur, K.; Mankala, R.; Chhabra, B. R. **2007**. Chemical modifications of DHCL from *Saussurea lappa* and the study of structure-activity relationship. *Indian Journal of Chemistry* **46B**, 847-851.
87. Yuuya, S.; Hagiwara, H.; Suzuki, T.; Ando, M.; Yamada, A.; Suda, K.; Kataoka, T.; Nagai, K. **1999**. Guaianolides as Immunomodulators. Synthesis and Biological Activities of Dehydrocostus Lactone, Mokko Lactone, Eremanthin, and Their Derivatives. *Journal of Natural Products* **62***,* 22-30.
88. Cantrell, C. L.; Nunez, I. S.; Castaneda-Acosta, J.; Foroozesh, M.; Fronczek, F.R.; Fischer, N. H.; Franzblau, S. G. **1998**. Antimycobacterial Activities of Dehydrocostus Lactone and Its Oxidation Products. *Journal of Natural Products* **61**, 1181-1186.
89. Hussain, S.; Tripathi, D.; Sharma, M. **2011**. Synthesis and Biological Study of Some New Derivatives of Sesquiterpene Lactones Isolated from Medicinal Plants. *Journal of Physical Science* **22**, 57–75.
90. Wedge, D.E.; Galindo, J.C.; Macías, F.A. **2000**. Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. *Phytochemistry* **53**, 747–757.

91. De Luque, A. P.; Galindo, J.C.G.; Macías, F.A.; Jorrín, J. **2000**. Sunflower sesquiterpene lactone models induce Orobanche Cumana seed germination. *Phytochemistry* **53**, 45–50.
92. Kupchan, S.M.; Eakin, M.A.; Thomas, A.M. **1971**. Tumor Inhibitors. 69. Structure-cytotoxicity relations among the sesquiterpene lactones. *Journal of Medicinal Chemistry* **14**, 1147–1152.
93. Rodriguez, E.; Towers, G.N.H.; Mitchell, J.C. **1976**. Biological activities of sesquiterpene lactones. *Phytochemistry* **15**, 1573–1580.
94. Klayman, D. L. **1993**. "*Artemisia annua*: from weed to respectable antimalarial plant," in Human Medicinal Agents from Plants, A. D. Kinghom and M.F. Balandrin, Eds. *American Chemical Society Symposium Series*, pp. 242–255, Washington, DC,USA.
95. Mohamed, A.E.H.; El-Sayed, M.A.; Hegazy, M.E.; Helaly, S.E.; Esmail, A.M.; Mohamed, N.S. **2010**. Chemical constituents and biological activities of *Artemisia herba-alba*. *Records of Natural Products* **4**, 1-25.
96. Brown, G. D. **2010**. The Biosynthesis of Artemisinin (Qinghaosu) and the Phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules* **15**, 7603-7698.
97. Abad, M. J.; Bedoya, L. M.; Apaza, L.; Bermejo, P. **2012**. The *Artemisia* L. Genus: A Review of Bioactive Essential Oils. *Molecules* **17**, 2542-2566.
98. Tzenkova, R.; Kamenarska, Z.; Draganov, A.; Atanassov, A. **2010**. Composition of *Artemisia Annua* Essential Oil Obtained from Species Growing Wild in Bulgaria. *Biotechnology and Biotechnological Equipment* **24**, 1833-1835.
99. Rimada, R., S.; Gatti, W.O.; Jeandupeux,R.; Cafferata, F.R. **2009**. Isolation, characterization of Artemisinin by NMR from Argentinean *Artemisia annua* L. *Bol Latinoam Caribe Plant Medicine and Aromatics* **8**, 275-281.
100. Garcia, L.C. **2015**. A Review of *Artemisia annua* L.: Its Genetics, Biochemical Characteristics, and Anti-Malarial Efficacy. *International Journal of Science and Technology* **5**, 38-46.
101. Brown, G. D.; Sy, L-K. **2004**. *In vivo* transformations of dihydroartemisinic acid in *Artemisia annua* plants. *Tetrahedron* **60**, 1139–1159.

102. Edwards, S.; Mesfin, T.; Hedberg, I. (eds). **1995**. Flora of Ethiopia and Eritrea. Vol. **2**, No. 2. The National Herbarium, Addis Ababa and Department of Systematic Botany, Uppsala, Et/SE, pp100.
103. Batish, D. R.; Singh, H. P.; Kohli, R. K.; Kaur, S. **2008**. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management* **256**, 2166–2174.
104. Asefa, A.; Dagne, E. **1997**. Essential Oils of Three *Eucalyptus* Species Acclimatized in Ethiopia. *Bulletin of Chemical Society of Ethiopia* **11**, 47-50.
105. Muchoril, P.; Mang'urol, L.; ChikamajI, B.; Dagne, E.; Bekele, T. **1997**. Essential oils of five Eucalyptus species grown in Kenya. *SINET: Ethiopian Journal of Science* **20**, 139-143.
106. Ryding, O.; Hedberg, I.; Ensermu, K.; Edwards, S.; Sebsebe, D.; Persson, E. **2006**. Flora of Ethiopia and Eritrea. Vol. **5**.The National Herbarium, Addis Ababa and Department of Systematic Botany, Uppsala, Et/SE, Pp, 516,552-553,560.
107. Dagne, E.; Hailu, S.; Bisrat, D.; Worku, T. **1997**. Constituents of the Essential Oil of *Thymus schimperi*. *Bulletin of Chemical Society of Ethiopia* **12**, 79-82.
108. Baser, K.H.C.; Ozek, T.; Kirimer, N.; Malyer, H. **1993**. The Essential Oil of *Thymus bornmuelleri* Velen. *Journal of Essential Oil Research* **5**, 691-692.
109. Baser, K. H. C.; Kurkcuoglu, M.; Ozek, T.; Tumen, G.; AKgul, A. **1995**. Essential Oil of *Thymus sipyleus* Boiss. Subsp. *sipyleus* var. *sipyleus*. *Journal of Essential Oil Research* **7**, 411-413.
110. Baser, K.H.C.; Demirci, B.; Kurkcuoglu, M.; Tumen, G. **1999**. Essential Oil of *Thymus zygioides* Griseb. Var. *zygioides* from Turkey. *Journal of Essential Oil Research* **11**, 409-410.
111. Santhanam, S. R.; Subramanian, M.; Egigu, M. C.; Parida, A. **2014**. Pentacyclic Triterpenoids and a Linear Alkane from the Milky Mangrove Tree (*Excoecaria agallocha* L.) are toxic to the larva of *Helicoverpa armigera* Hubner. (Lepidoptera: Noctuidae). *International Journal of Advanced Research* **2**, 1-12.

112. Ayatollahi, S.A.M.; Kobarfard, F.; Asgarpanah. J.; Ahmed, Z. **2007**. Chemical constituents from *Otostegia persica*. *Journal of Chemical Society of Pakistan* **29**, 61-63.

113. Koay, Y. C.; Wong, K. C.; Osman, H.; Eldeen, I. M. S.; Asmawi, M. Z. **2013**. Chemical Constituents and Biological Activities of *Strobilanthes crispus* L. *Records of. Natural Product* **7**, 59-64.

114. Lee, H.T; Yang, S.W; Kim, K.H; Seo, E.K; Mar, W. **2002**. Pseudoguaianolides isolated from *Inula britannica var. chinensis* as inhibitory constituents against inducible nitric oxide synthase. *Archives of Pharmacal Research* **25**,151–153.

115. Bohlmann, F.; Mahanta.P. K. **1979**. Zwei Neue Pseudoguajanolide Aus *Telekia Speciosa*. *Phytochemistry*, 887-888.

116. Feng, J-T; Ma, Z-Q; Li, J-H; He, J.; Xu, H.; Zhang, X. **2010**. Synthesis and Antifungal Activity of Carabrone Derivatives. *Molecules* **15**, 6485-6492.

117. Fontana, G.; La Rocca, S.; Passannanti, S.; Pia Paternostro, M. **2007**. Sesquiterpene compounds from *Inula viscosa*. *Natural Product Research* **21**, 824-827.

118. Wu, J.; Tang, C.; Chen, L.; Qiao, Y.; Geng, M.; Ye, Y. **2015**. Dicarabrones A and B, a Pair of New Epimers Dimerized from Sesquiterpene Lactones via a [3 + 2] Cycloaddition from *Carpesium abrotanoides*. *Organic letters* **17**, 1656–1659.

119. Wang, D.; Ren, S.; Wang, H.; Yan, H.; Feng, J.; Zhang, X. **2014**. Semisynthesis and Antifungal Activity of Novel Oxime Ester Derivatives of Carabrone Modified at C (4) against *Botrytis cinerea*. *Chemistry and biodiversity* **11**, 886-903.

120. Wang, H.; Ren, S-X.; He, Z-Y.; Wang, D-L.; Yan, X-N.; Feng, J-T.; Zhang, X. **2014**. Synthesis, Antifungal Activities and Qualitative Structure Activity Relationship of Carabrone Hydrazone Derivatives as Potential Antifungal Agents. *International Journal Molecular Science* **15**, 4257-4272.

121. Maruyama, M.; Omura, S. **1977**. Carpesiolin from *Carpesium abrotanoides. Phytochemistry* **16**, 782–783.

122. Wu, Z-J.; Shan, L.; Lu, M.; Shen, Y-H; Tang, J.; Zhang, W-D. **2010**. Chemical constituents from *Inula cappa*. *Chemistry of Natural Compounds* **46***,* 298-300*.*

123. Yang, H. H.; Son, J-K.; Jung, B.; Zheng, M. S.; Kim, J-R. **2011**. Epifriedelanol from the Root Bark of *Ulmus davidiana* Inhibits Cellular Senescence in Human Primary Cells. *Planta Medica* **77**,441–449.

124. Bianchini, J-P; Gaydou, E. M.; Rafaralahitsimba, G.; Waegell B.; Zahra, J. **1998**. Dammarane Derivatives in the Fruit Lipids of *Olea madagascariensis. Phytochemistry* **27**, 2301-2304.

125. Mossa, J.S.; El-Feraly, F. S.; Muhammad, I.; Zaw, K.; Mbwambo, Z.H.; Pezzuto, J. M.; Fong, H.H.S. **1997**. Sesquiterpene Lactones and Thymol Esters from *Vicoa pentanema. Journal of Natural Products* **60**, 550-555.

126. Cho, K-M.; An, X-H.; Chon, J-K.; Kim, H-S.; Chun, J-C. **2010**. Foliage Contact Herbicidal Activity of Dehydrocostus lactone derived from *Saussurea lappa*. *Korean Journal of Weed Science* **30** (4), 421-428.

127. Mathur, S.B.; Hiremath, S. V.; Kulkarni, G. H.; Kelka, G.R; Bhattacharyya S.C.; Simonovic, D. **1965**. Terpenoids-LXX: Structure of dehydrocostus lactone. *Tetrahedron* **21**, 575–590.

128. Blaskó, G.; Cordel, G. A. I; Lankin, D. C. **1988**. Definitive ^{1}H- and ^{13}C-NMR Assignments of Artemisinin (Qinghaosu). *Journal of Natural Products* **51** (6), 1273–1276.

129. Darmawan, A.; Kosela, S.; Kardono, L.B.S.; Syah, Y. M. **2012**. Scopoletin, a coumarin derivative compound isolated from *Macaran gagigantifolia* Merr. *Journal of Applied Pharmaceutical Science* **2** (12),175-177

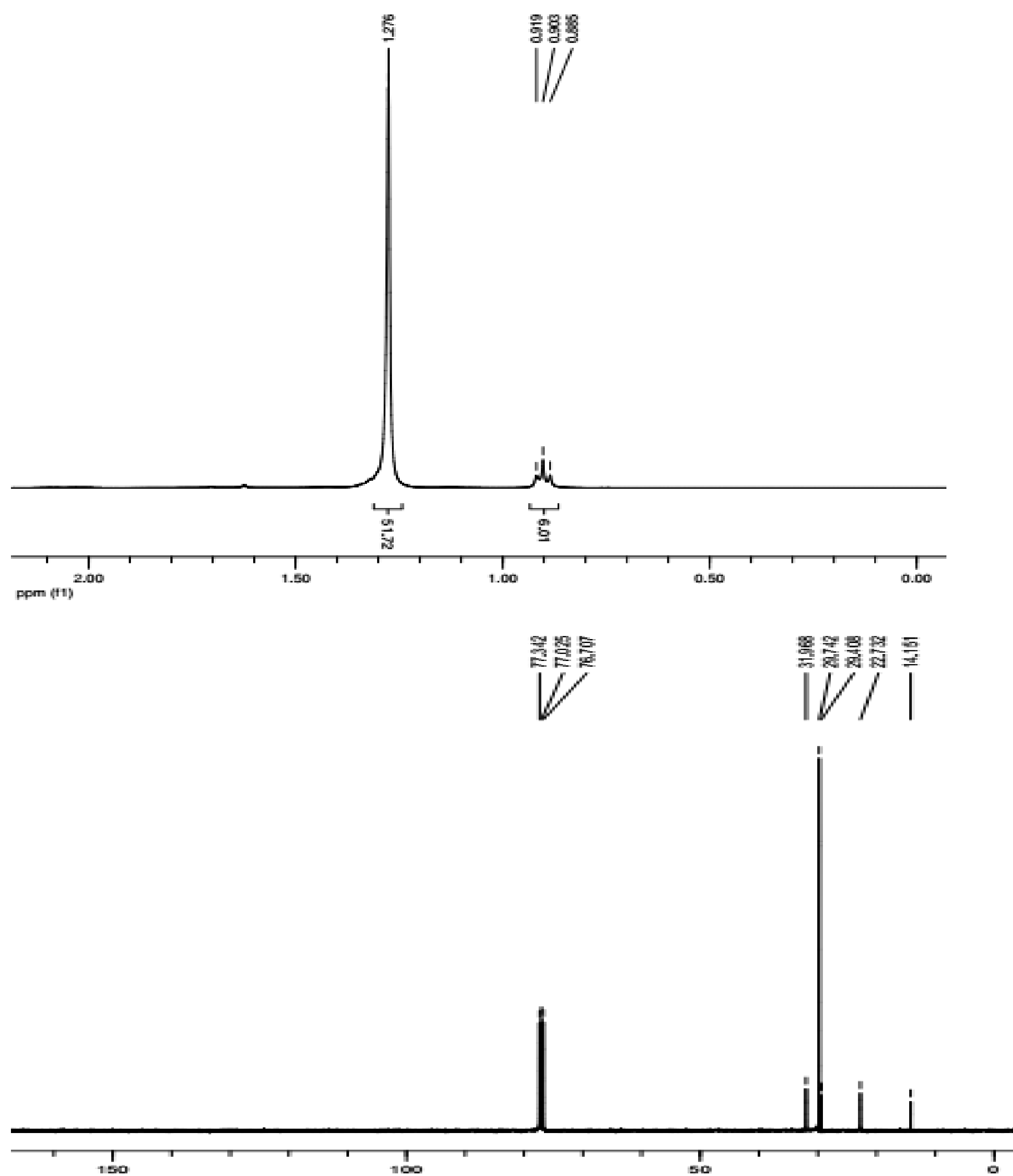
130. Wallaart, T. E.; Uden, W-V; Lubberink, H. G. M.; Woerdenbag, H. J.; Pras,N.; Quax W. J. **1999**. Isolation and Identification of Dihydroartemisinic Acid from *Artemisia annua* and its possible role in the Biosynthesis of Artemisinin. *Journal of Natural Products* **62***,* 430-433.

131. Misra, L.N.; Ahmad, A.; Thakur, R.S. **1993**. Crystal structure of artemisinic acid: a possible biogenetic precursor of antimalarial artemisinin from *Artemisia annua*. *Journal of Natural Products* **56**, 215-219.

132. Gakuubi, M. M.; Wanzala, W.; Wagacha, J. M.; Dossaji, S. F. **2016**. Bioactive properties of *Tagetes minuta* L. (Asteraceae) essential oils: A review. *American Journal of Essential Oils and Natural Products* **4**, 27-36.
133. Das, K.; Tiwari, R.K.S.; Shrivastava, D. K. **2010**. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends: Review. *Journal of Medicinal Plants Research* **4**, 104-111.
134. Ambikapathy, V.; Gomathi, S.; Panneerselvam, A. **2011**. Effect of antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse). *Asian Journal of Plant Science and Research* **1**,131-134.
135. Scorzoni, L.; Benaducci, T.; Fusco Almeida, A.M.; Siqueira Silva, D.H.; Silva Bolzani, V. da M.; Mendes Gianinni, J. S. **2007**. The Use of Standard Methodology for Determination of Antifungal Activity of Natural Products against Medical Yeasts *Candida* Sp. and *Cryptococcus* spp. *Brazilian Journal of Microbiology* **38**,391-397.
136. Appendino, G.; Calleri, M.; Chiari, G. **1986**. Structure of Graveolide and conformational aspects of related helenanolodes. *Gazzetta Chimica Italiana* **116**, 637-641.
137. Minato, H.; Horibe, I. **1968**. Studies on Sesquiterpenoids. Part XVIII. Total Synthesis of Carabrone. *Journal of the Chemical Society* (C), 2131-2137.
138. Minato, H.; Nosaka, S.; Horibe, I. **1964**. Studies on Sesquiterpenoids. Part VIII. The Structure of Carabrone, a New Component of *Carpesium abrotanoides*, Linn. *Journal of the Chemical Society* (C), 5503-5510.
139. Escalante, J.; Carrillo-Morales, M.; Linzaga, I. **2008**. Michael Additions of Amines to Methyl Acrylates Promoted by Microwave Irradiation. *Molecules* **13***,* 340-347.
140. Mori, A.; Miyakawa, Y.; Ohashi, E.; Haga, T.; Maegawa, T.; Sajiki, H. **2006**. Pd/C-Catalyzed Chemoselective Hydrogenation in the Presence of Diphenylsulfide. *Organic Letters* **8**, 3279-3281.

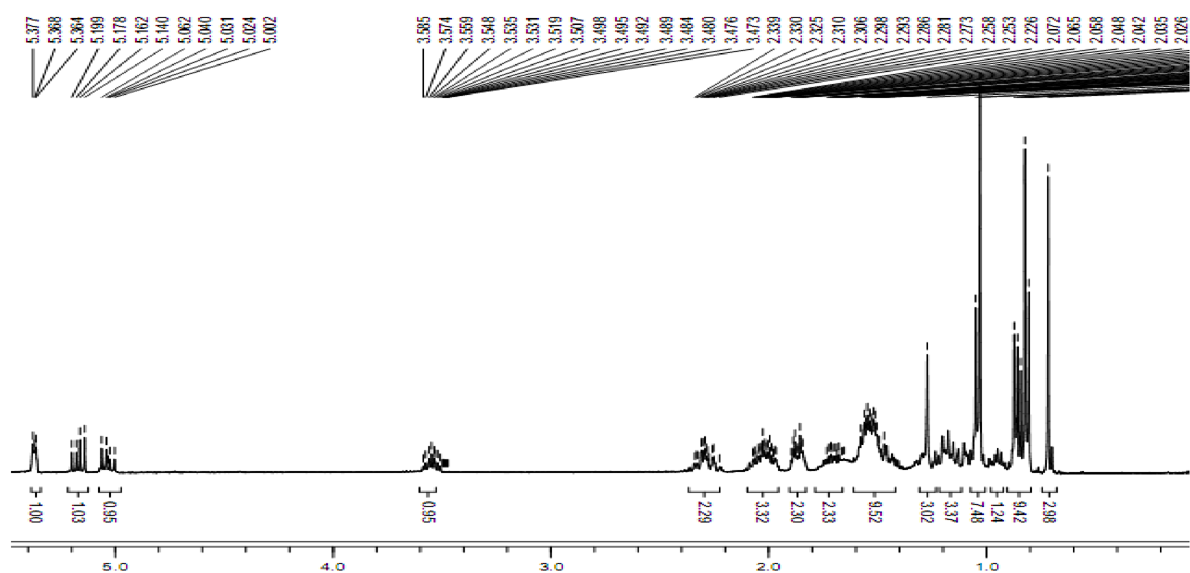
Appendix: NMR spectra of compounds

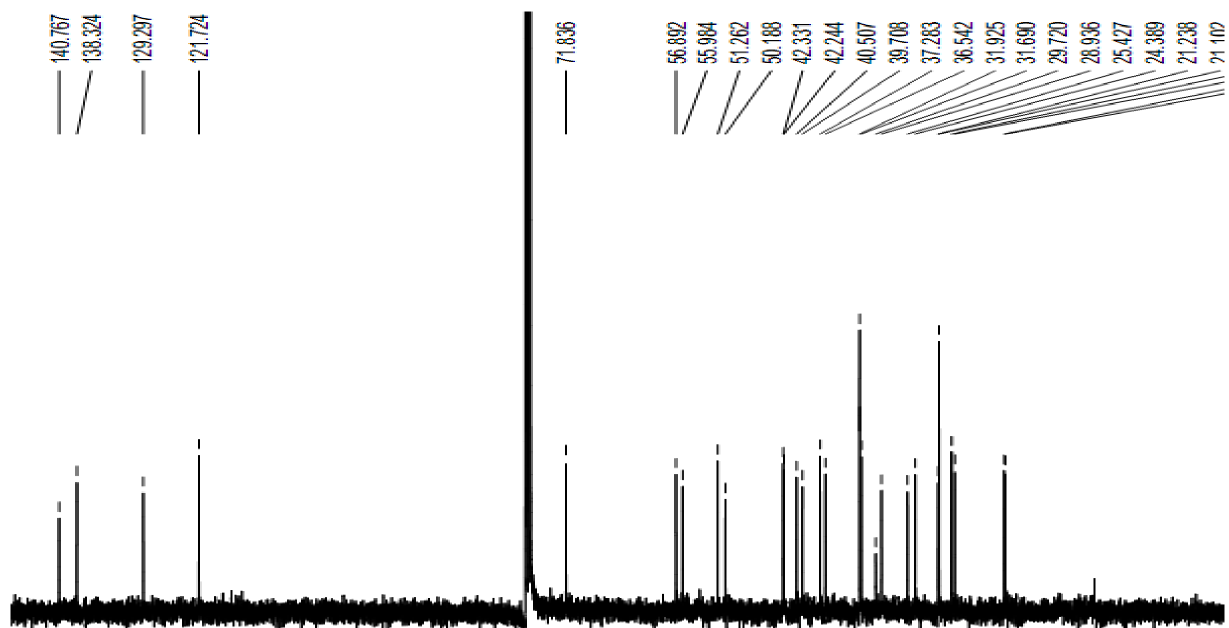
1. Compound **476** (96-40A)



2. Compound **306** (96-33I or 96-46Q)

3. Compound **307** (96-43Aor 96-35B or 96-59Q)



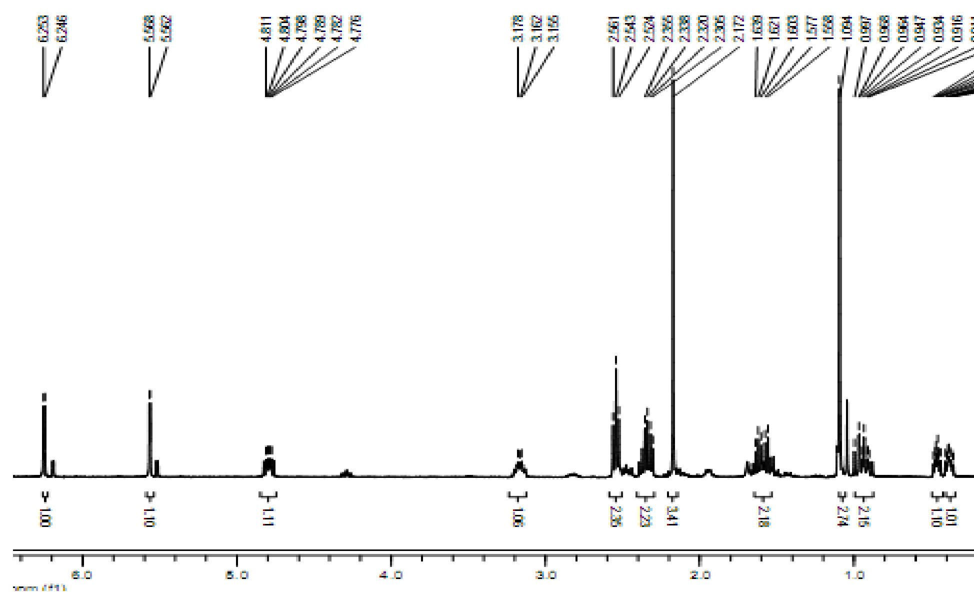


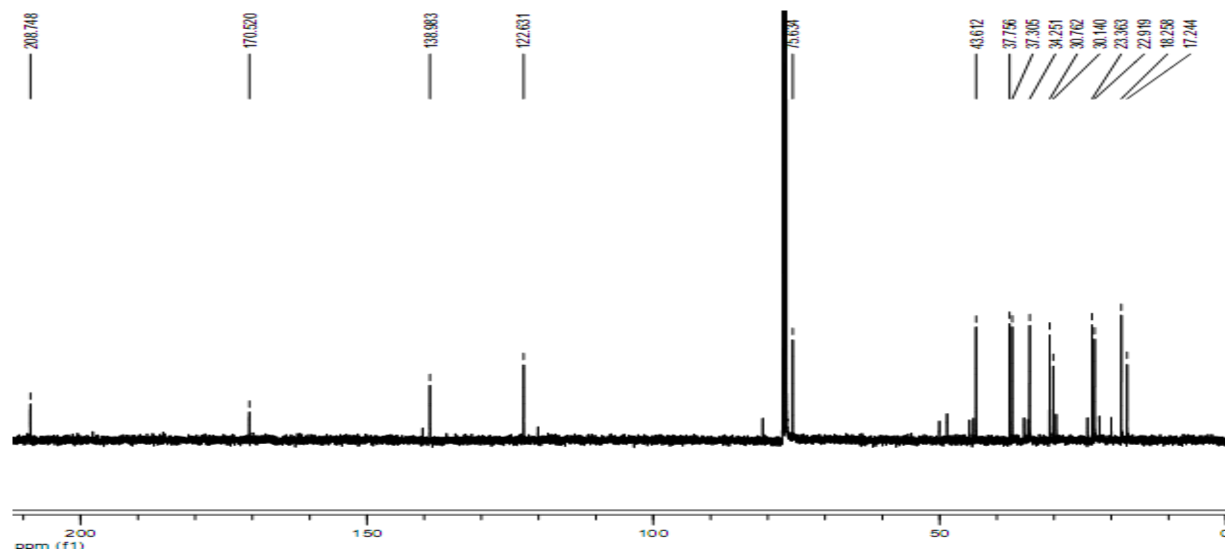
4. Compound **131** (96-31A/23C or 96-51J or 96-57M)

96-31A, 1H

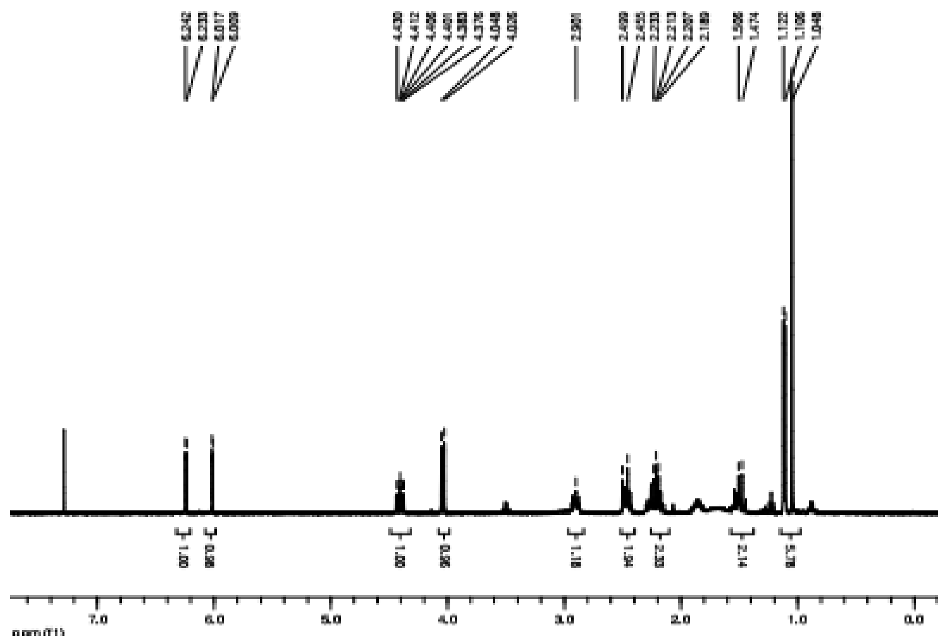
96-23C, CDCl3, C13, Nov 16, 2015

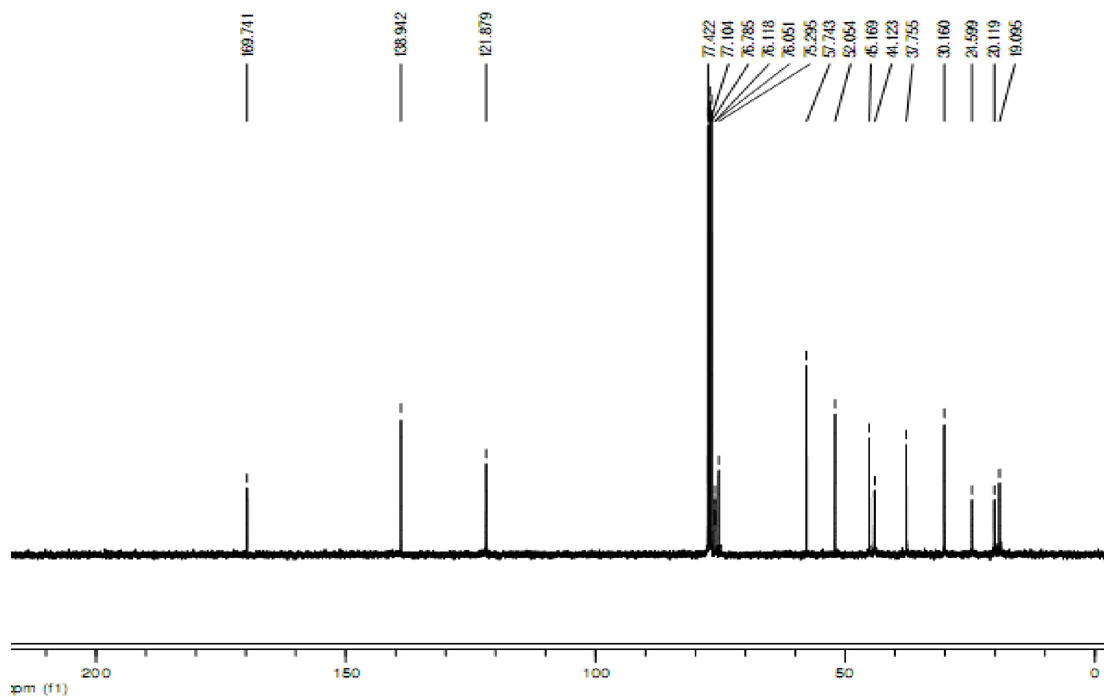
5. Compound **174** (96-32G or 96-35C or 96-57M)



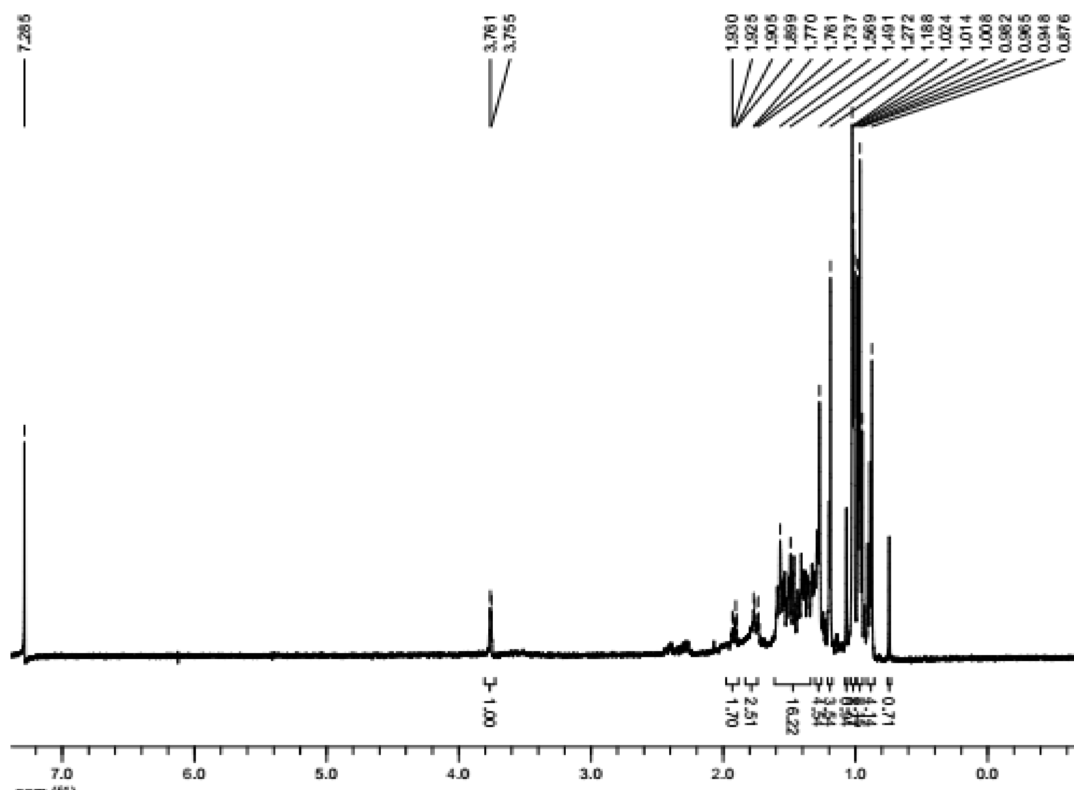


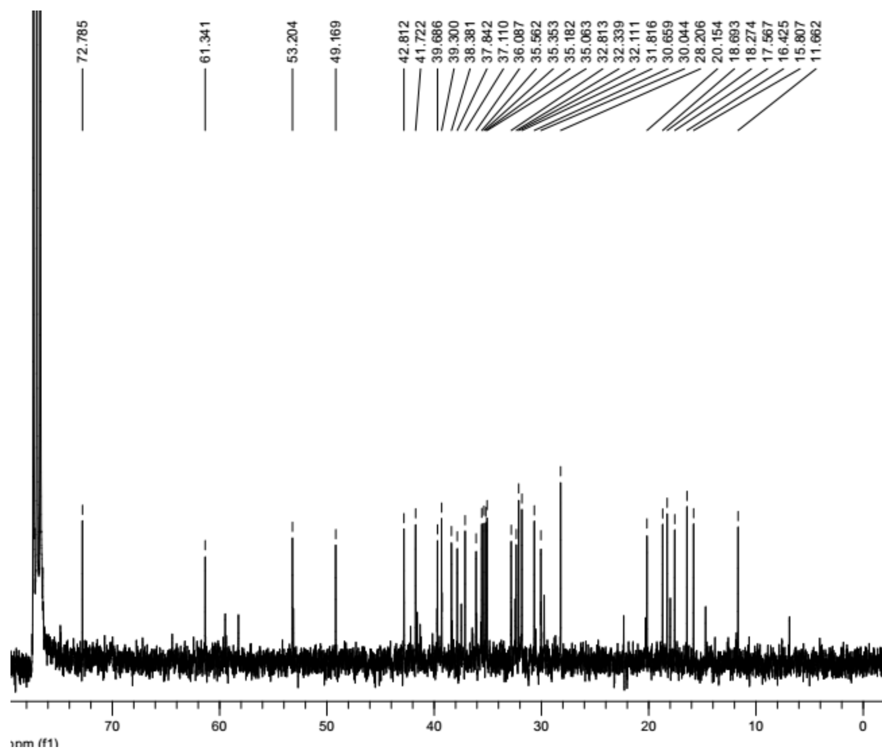
6. Compound **139** (96-45J or 96-51L or 96-57S)



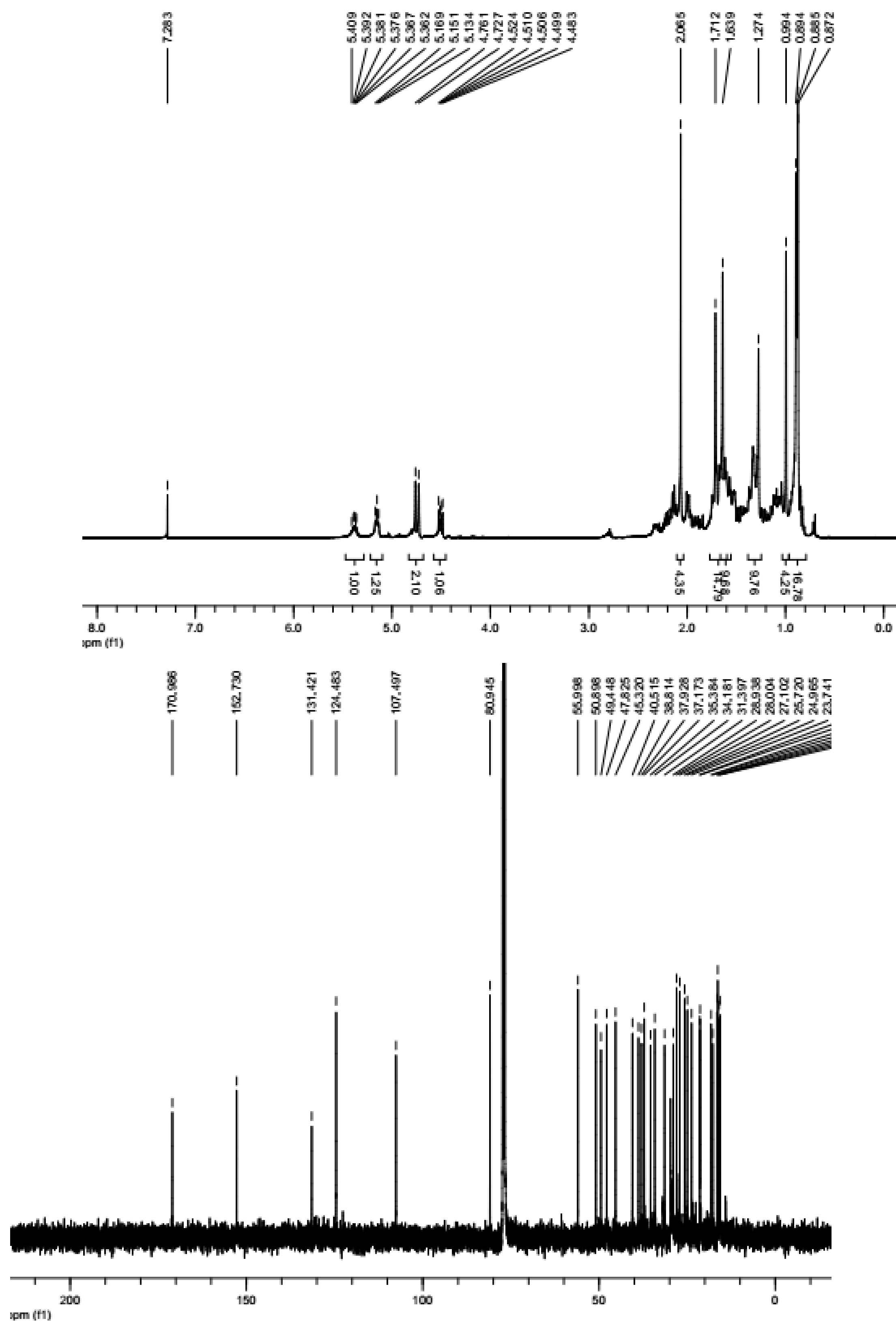


7. Compound **241** (96-39H)

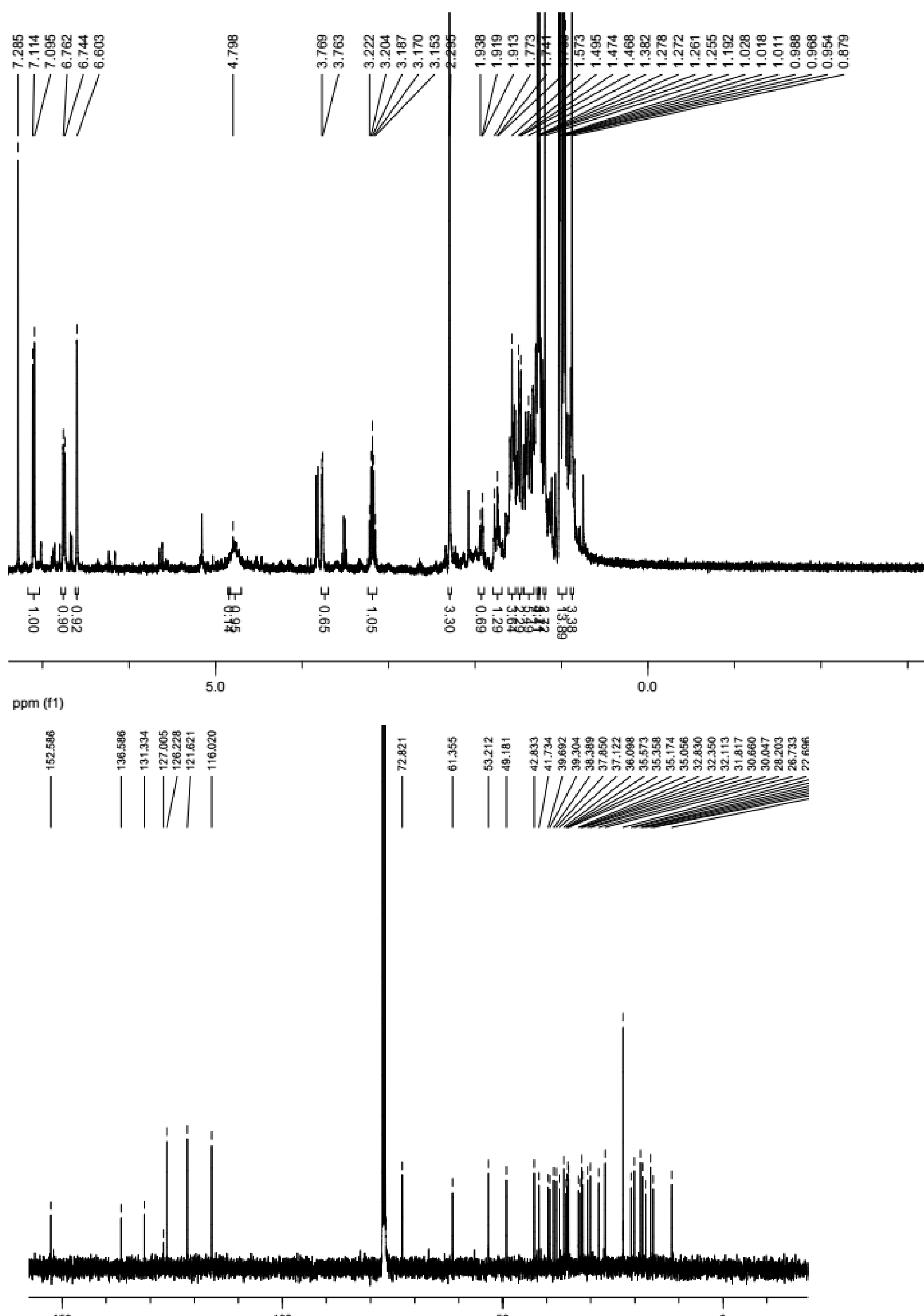




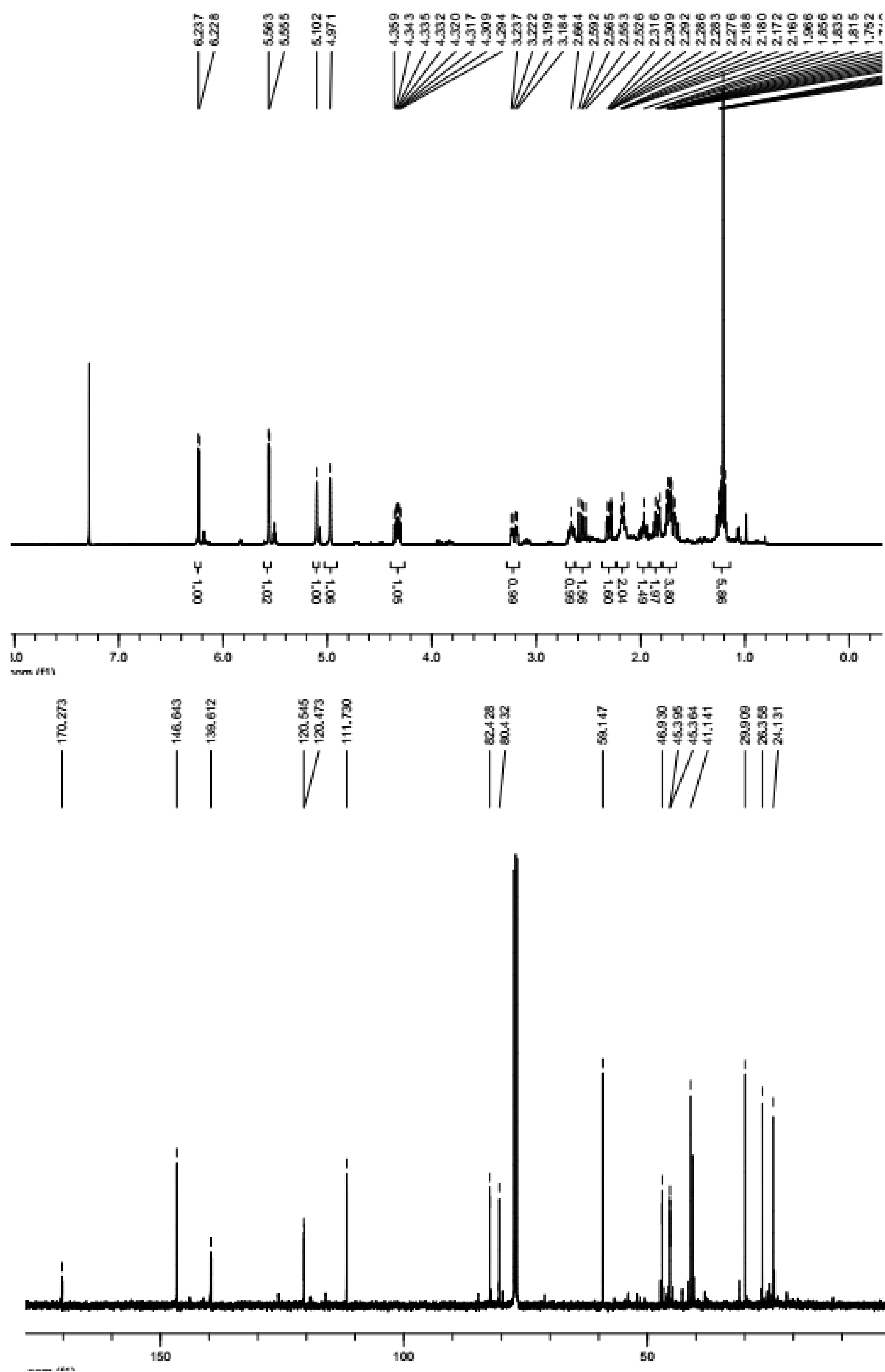
8. Compound **245** (96-56A)



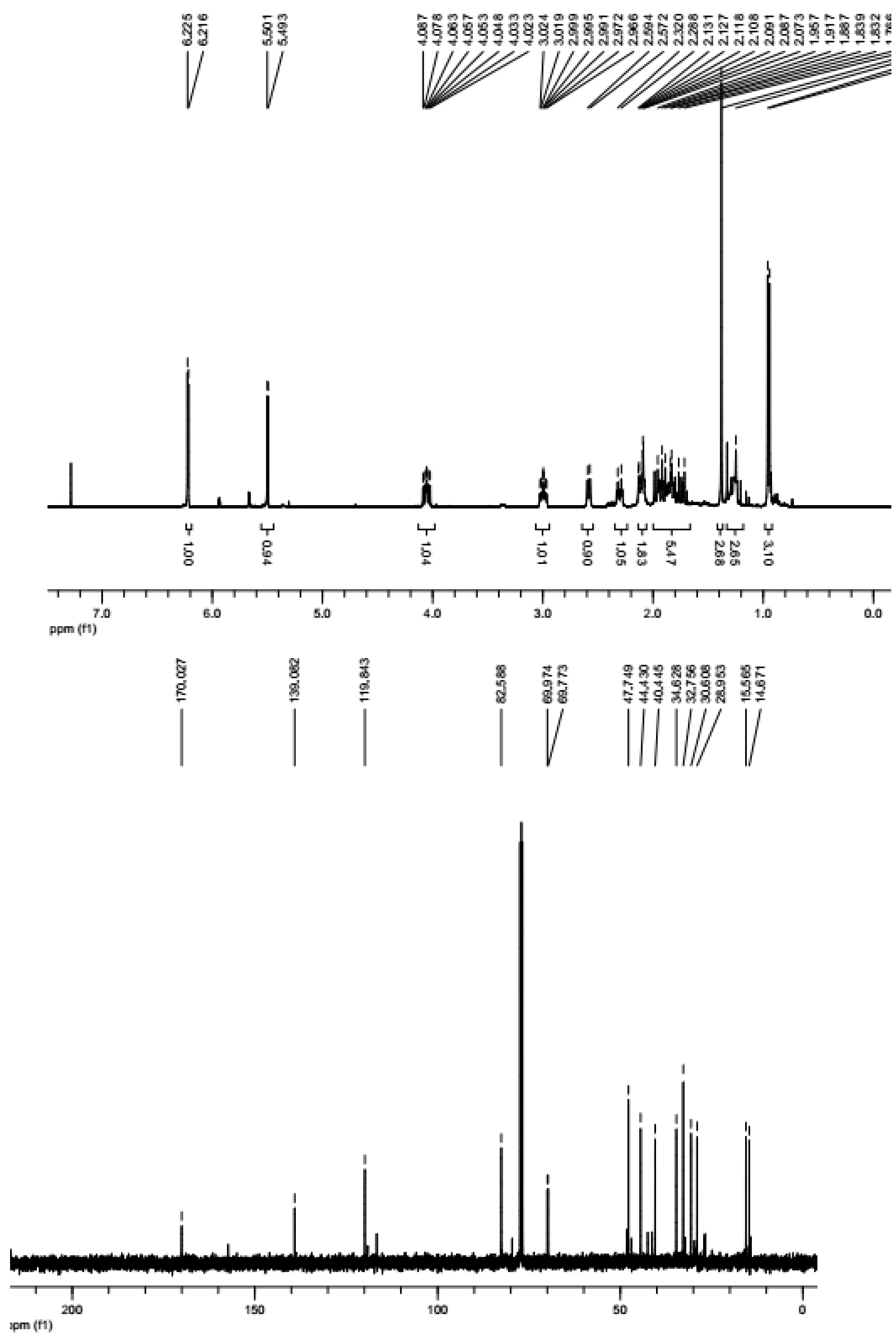
9. Compound **233 + 241** (96-48T)



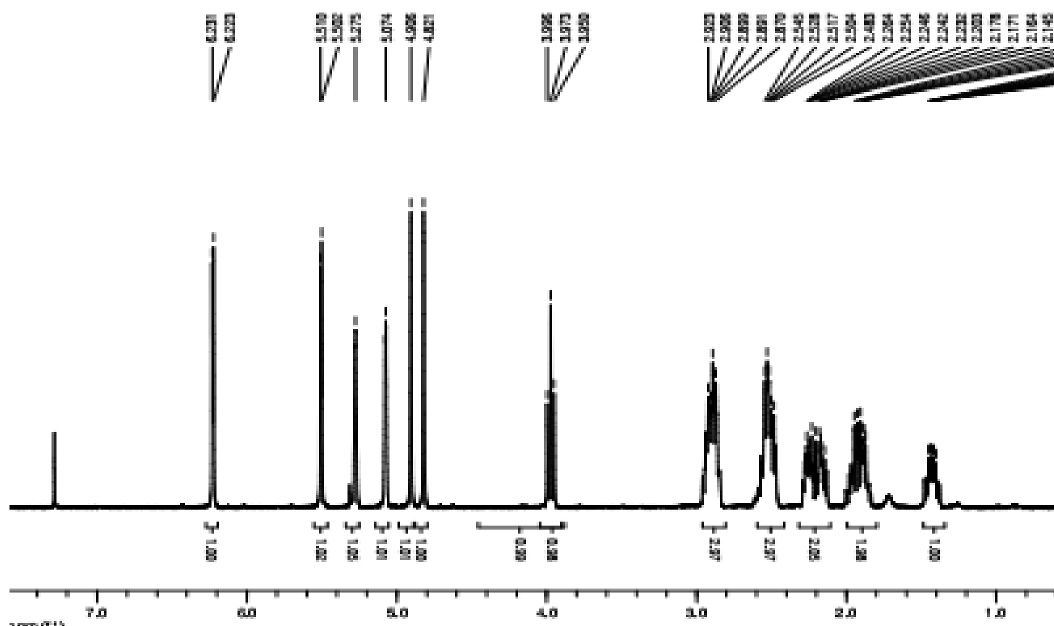
10. Compound **107** (96-59O)

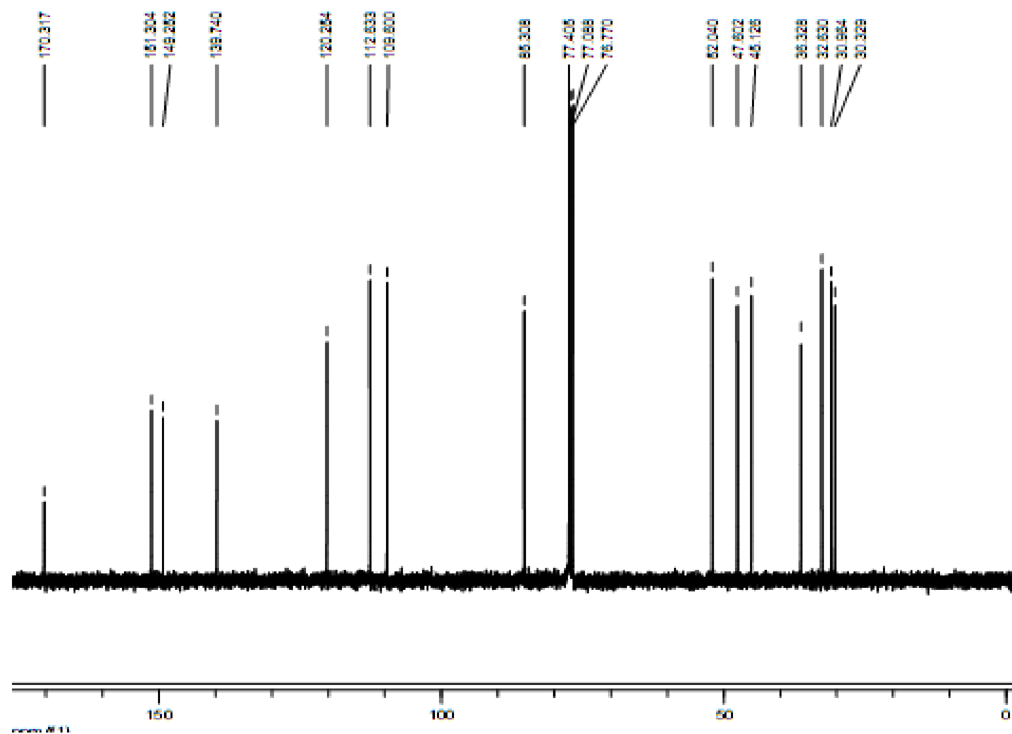


11. Compound **122** (96-59P)

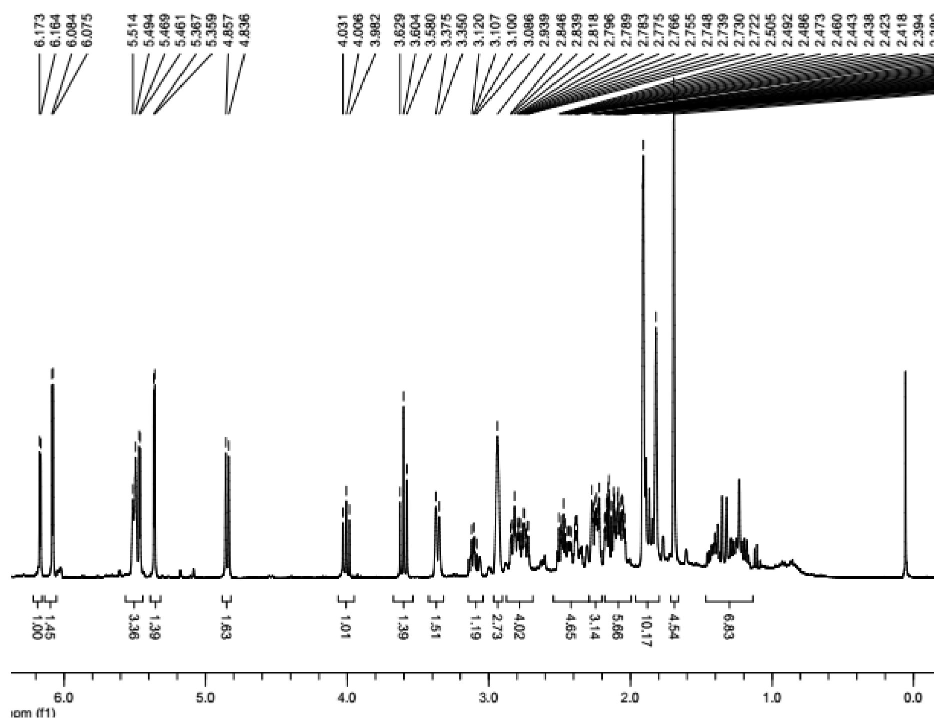


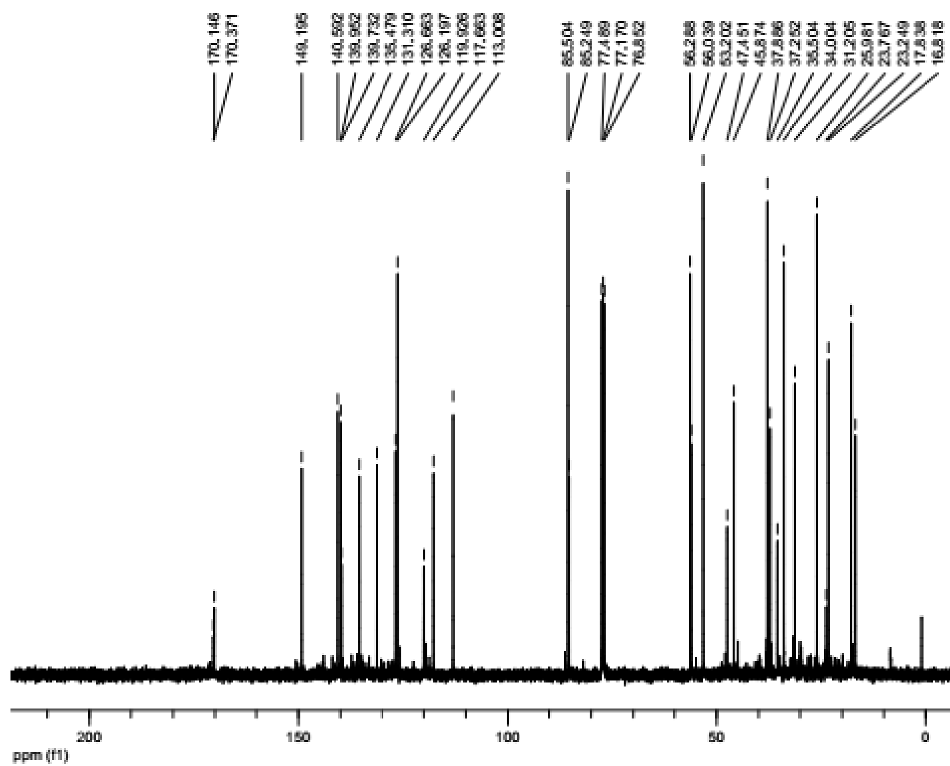
12. Compound **108 (**96-37D or 96-9K)



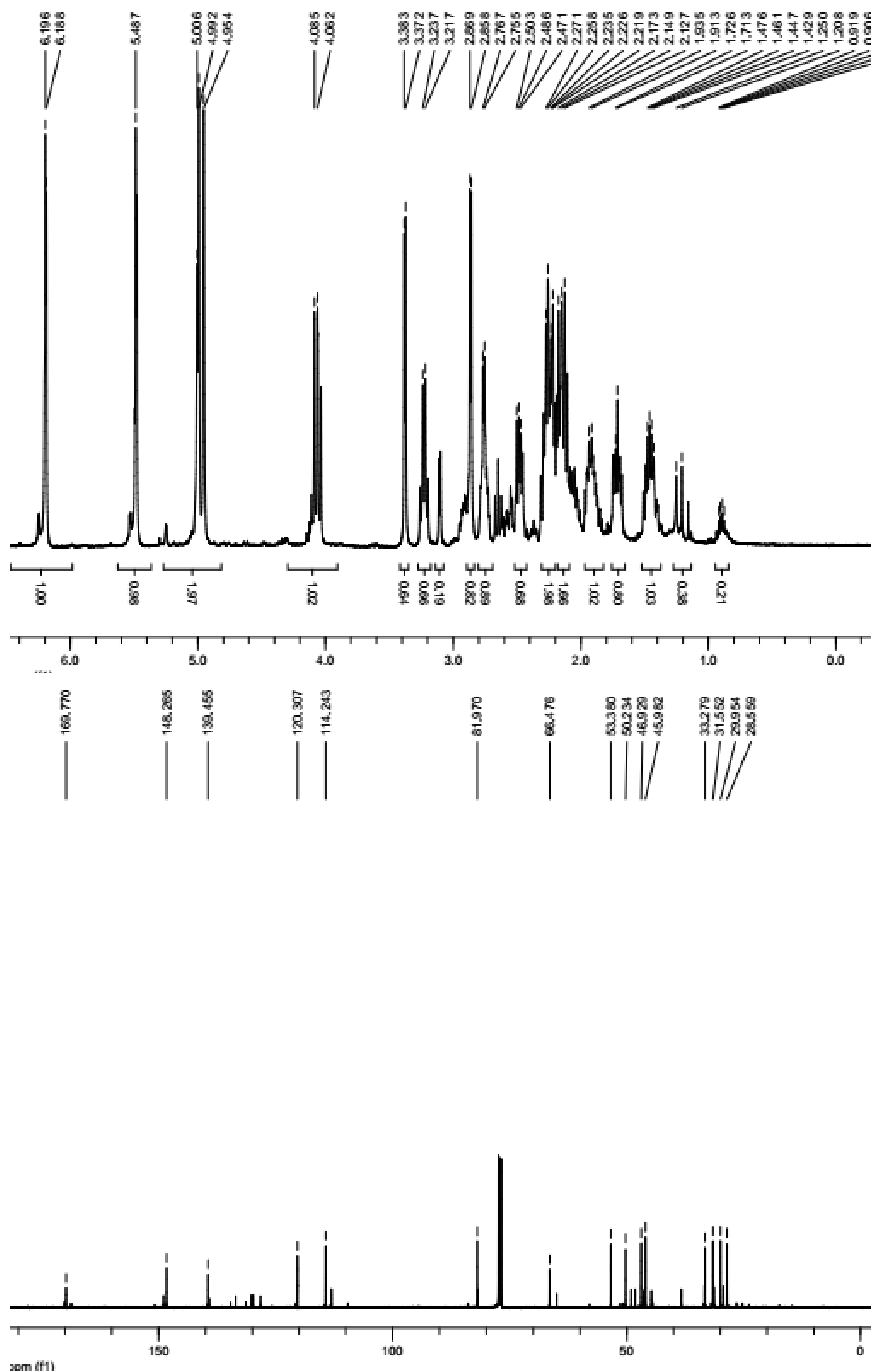


13. Compound **427/433** (96-56F)

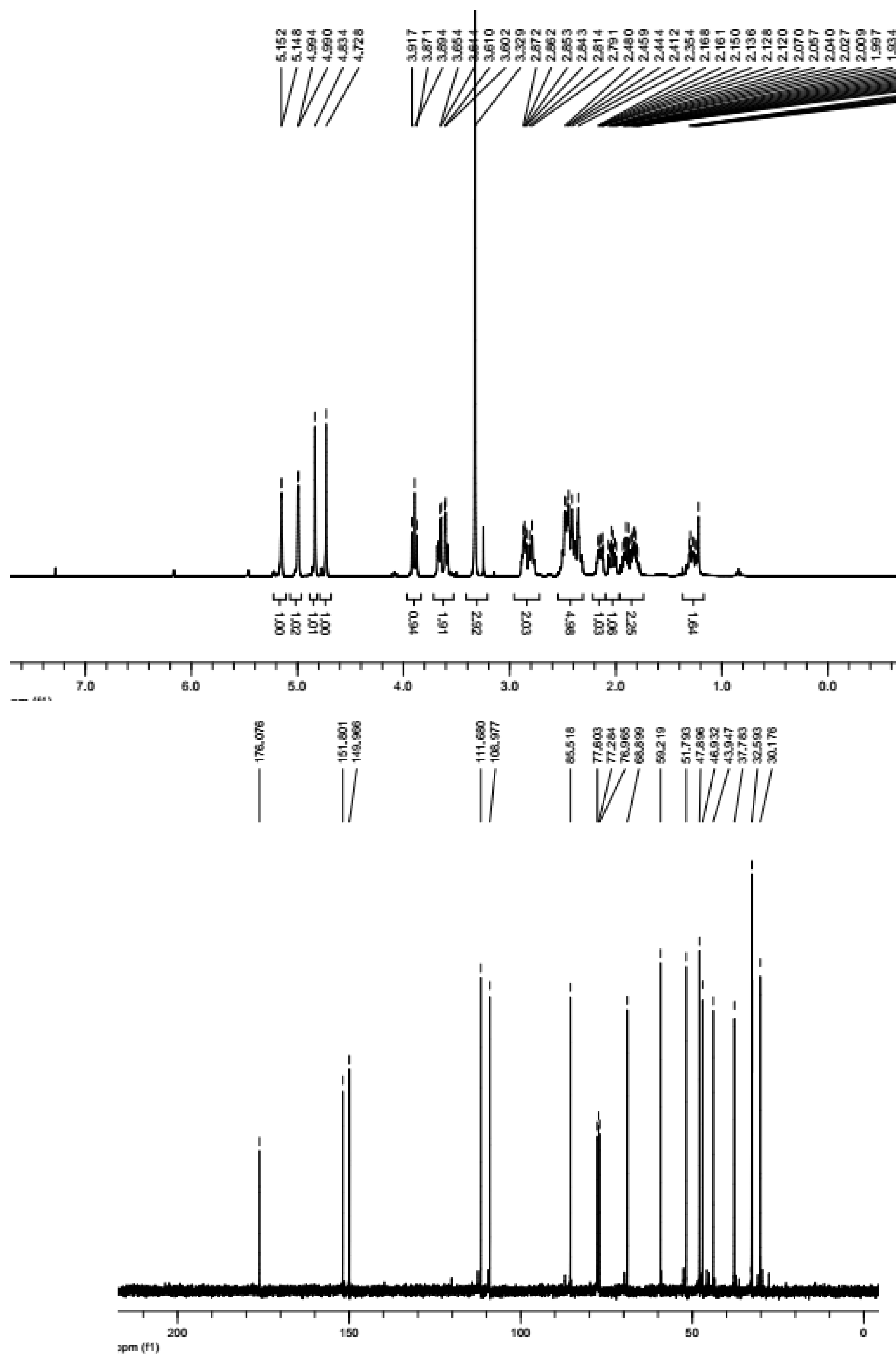




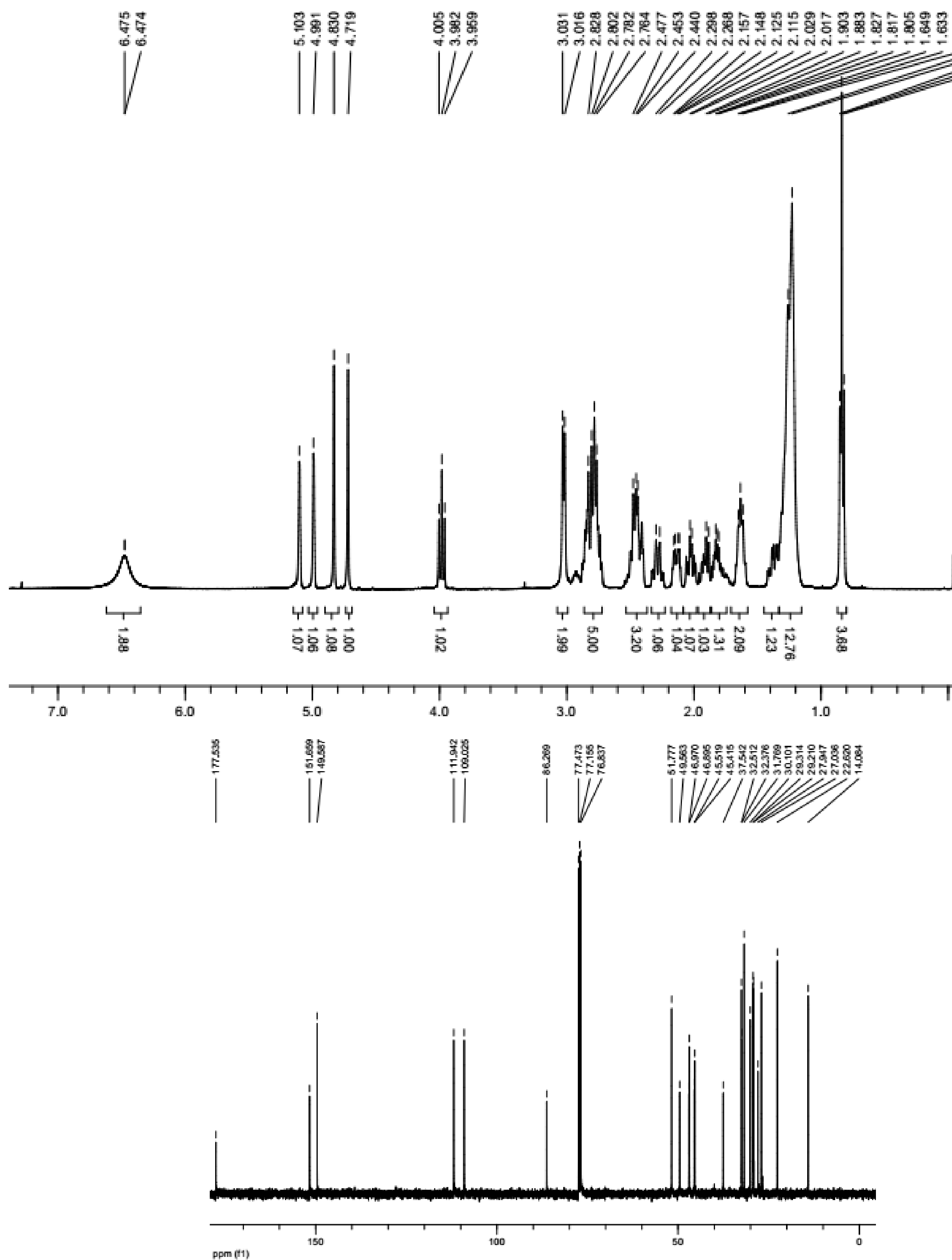
14. Compound **430** (96-60O)



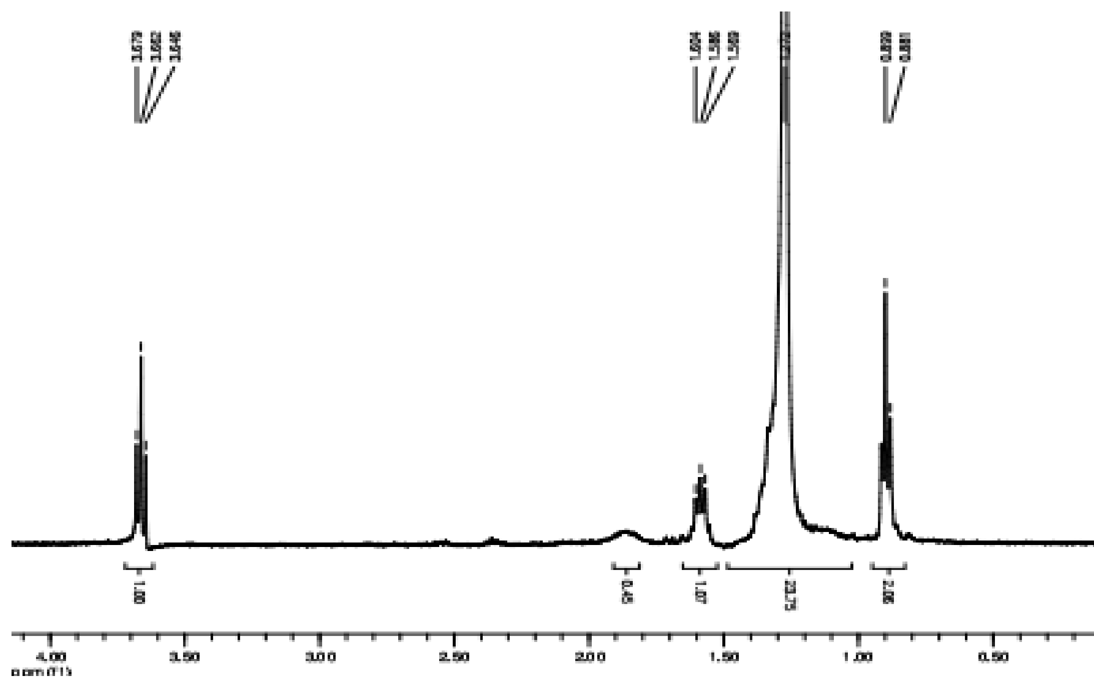
15. Compound **484** (96-56E or 96-60B)

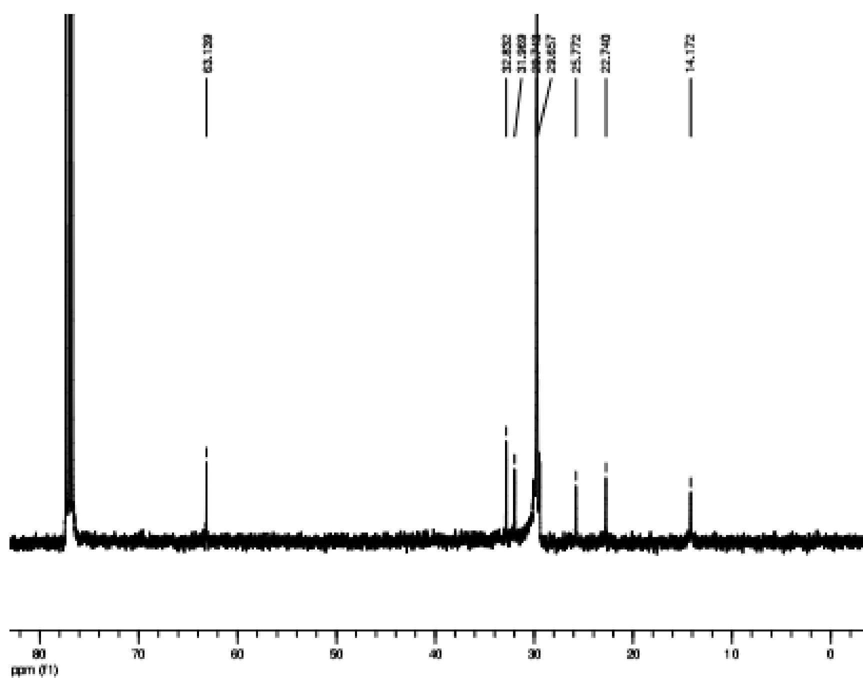


16. Compound **485** (96-62B)

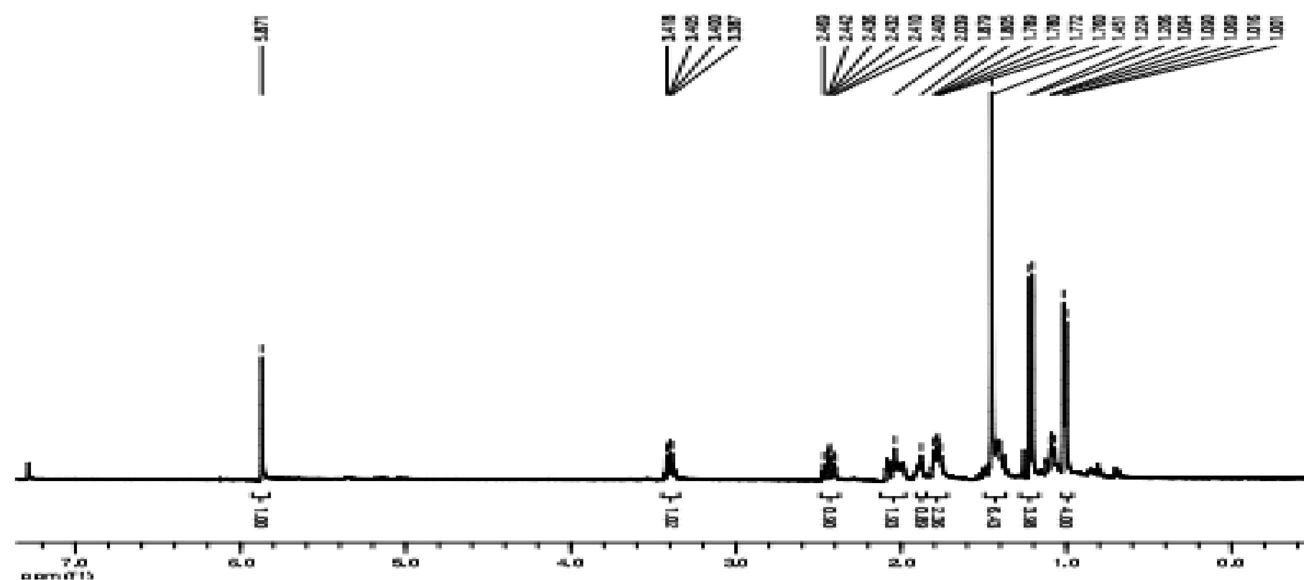


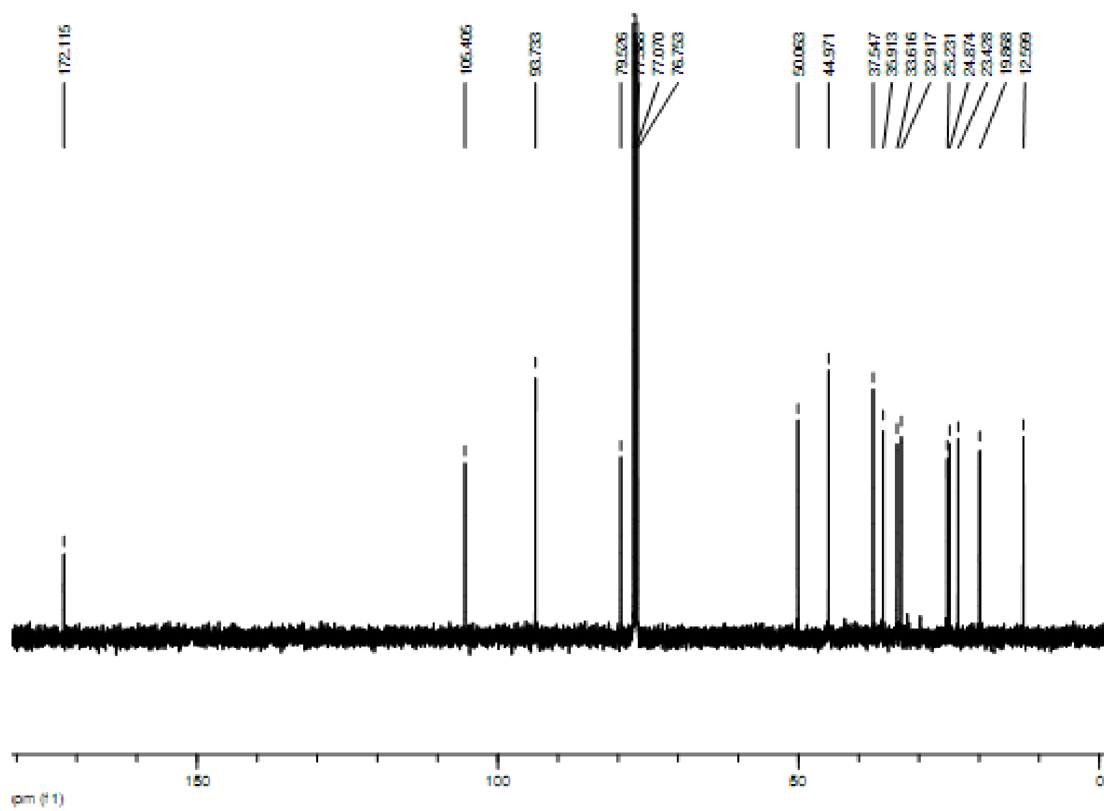
17. Compound **486** (96-50D)



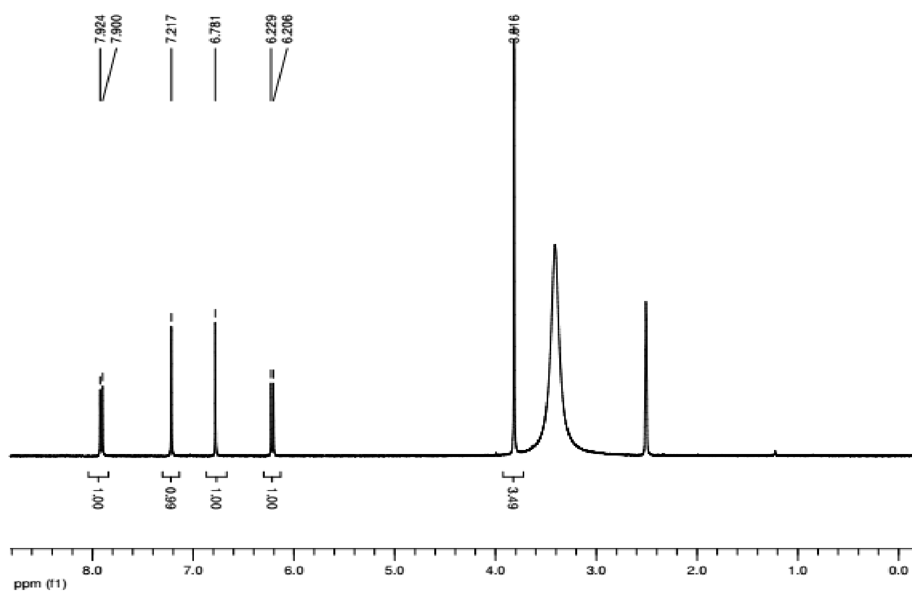


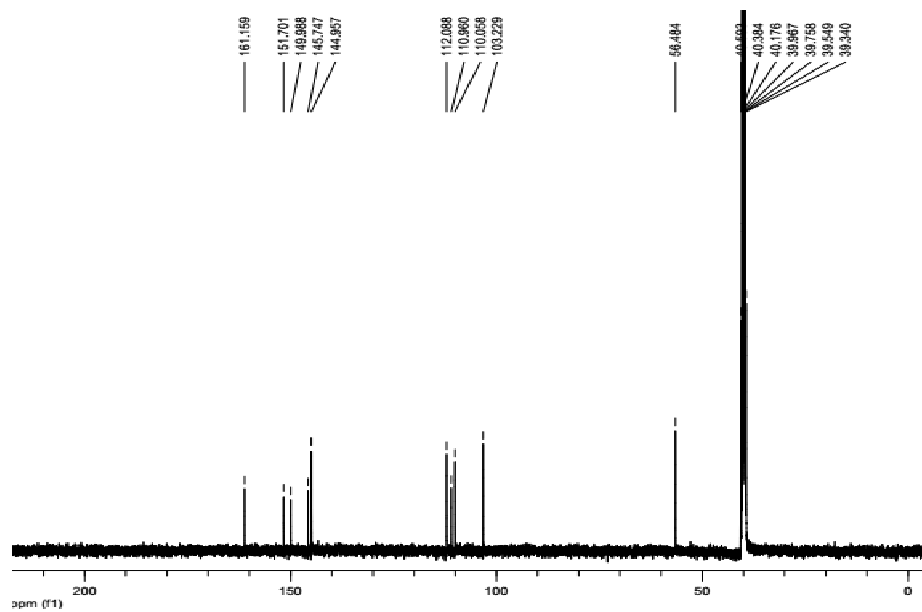
18. Compound **445** (96-50J)





19. Compund **404** (96-50P)





20. Compound **447/448** (96-60D)