PHYTOCHEMICAL EVALUATION OF ROOTS AND LEAVES EXTRACTS OF *OCIMUM CUFODONTII* FOR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

A MASTER THESIS SUBMITTED TO THE OFFICE OF GRADUATE STUDIES OF ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY, SCHOOL OF APPLIED NATURAL SCIENCE DEPARTMENT OF APPLIED CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED CHEMISTRY (MEDICINAL CHEMISTRY)

By

Muhdin Aliye

Advisor: Yadessa Melaku, PhD

Co-Advisor: Rajalakshmanan Eswaramoorthy, PhD

September, 2018

DECLARATION

I , undersigned, declare that this thesis is my original work and has not been presented for a degree or diploma in any other University. All sources or materials used for this thesis have been duly acknowledged.

Muhdin Aliye

Signature: _____

This thesis has been submitted for examination with my approval as University advisor and co-advisor.

Advisor: Yadesa Melaku, PhD

Signature: _____

Co-advisor: Rajalakshmanan Eswaramoorthy, PhD

Signature: _____

APPROVAL SHEET

This is to certify that the thesis entitled "Phytochemical evaluation of roots and leaves extracts of *Ocimum cufodontii* for antibacterial and antioxidant activities*"* submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Chemistry with specialization in Medicinal Chemistry of School of Applied Natural Science MSc program of Department of Applied Chemistry, Adama Science and Technology University and is a record of original research carried out by Muhdin Aliye (ID. No GSR/0183/09), under my supervision, and no part of the thesis has been submitted for any other degree or diploma in any other University.

Submitted by	Name	Signature	Date
	_____	_____	_____
Approved by	Major Advisor	Signature	Date
	_____	_____	_____
	Co- Advisor	Signature	Date
	_____	_____	_____
External Examiner		Signature	Date
_____		_____	_____
Internal Examiner		Signature	Date
_____		_____	_____
Head of Program		Signature	Date
_____		_____	_____
School Dean		Signature	Date
_____		_____	_____
PG Dean		Signature	Date
_____		_____	_____

ACKNOWLEDGEMENT

First and foremost, praises and thanks to Allah for being my strength. You have given me the power to believe in myself and follow my dreams. I could never have done this without the faith I have in you.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my main advisor, Dr Yadesa Melaku and co advisor, Dr Rajalakshmanan Eswaramoorthy. They both have been fantastic supervisors with unique knowledge of chemistry. Their dedication and keen interest above all their enormous attitudes to help their students have been solely and mainly responsible for my motivation to keep working. Their guidance, carefully monitoring and professional advice helped me largely to understand and accomplish this task.

I owe a deep sense of gratitude to all participants of the ASTU chemistry research group for an enjoyable social and working environment and their support and practical advice.

I also express a deep sense of gratitude to my uncle shek Abdella Jara for selecting the medicinal plant and encouragement during my work in plant collection.

Thanks to my sisters, Mahabuba Aliye and Zahara chiriksa, with all their family who offered invaluable support and humor over the years.

Finally, and most importantly, I would like to thank my lovely wife Meseret chiriksa for her support, encouragement, quiet patience and love. My father, Aliye Jara, receive my deepest gratitude for his faith, co-operation, understanding and constant encouragement which were the strength in carrying out the work successfully. Without you all, I most certainly would not be where I am today.

Adama, Sep 2018

Muhdin Aliye

ABSTRACT

Ocimum cufodontii (Lamiaceae) is an indigenous plant to Ethiopia used traditionally for the treatment of relieving pain, fever and inflammatory disorders. In view of its traditional uses and limited scientific report, the roots and leaves were extracted with hexane, EtOAc and MeOH which furnished 1%, 1.7 % and 2.3 % for the root and 1.8%, 2% and 2.5 % for the leaves respectively. The leaves were hydro-distilled using Clevenger type apparatus to afford 16.66% yellowish oil. The essential oil was also analyzed for its chemical constituents using GC-MS which gave eleven major compounds with the most abundant constituent found to be *β*-caryophyllene (31.3%). The presence of this compound in significant amount was likely responsible for anti-inflammatory activity. The leaves extract after silica gel column chromatography furnished betulinic acid (**39)**. On the other hand, compound **40**, **41**, **42** and **43** were isolated from the hexane extracts of the roots of *Ocimum cufodontii*. Structure elucidation was done using spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, GC-MS and LC-MS. The antibacterial properties of *Ocimum cufodontii* essential oil from the leaves and hexane, EtOAc and methanol extract of root and leaves was studied on the standard gram-negative bacteria against (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonae* ,*Salmonella typhi*) and gram-positive, *Staphylococcus aureus*. Results showed that the essential oil displayed zone of inhibition of 19.0 mm against *S. aureus*. This was significant compared with the positive control Chloroamphinicol which exhibited 28 mm zone of inhibition. On other hand, the hexane and MeOH extract of the root showed inhibition zone of 17 mm and 15 respectively. The antioxidant activities of *Ocimum cufodontii* was carried using DPPH (1, 1-diphenyl-2-picryl hydrazyl). Among the four fractions,the hexane root extract showed the highest scavenging activity, with an IC_{50} value of 1. 30 µg/mL. Besides, hexane leave extract with an IC_{50} value of 1.97µg/mL showed more efficient radical-scavenging abilities than ethyl acetate leave and root extract with an IC_{50} value of 55.83 µg/mL and 116.58 µg/mL respectively. The antibacterial and antioxidant activities observed in the present work corroborate the traditional use of this plant.

Keywords: Lamiaceae*, Ocimum, Ocimum cufodontii*, DPPH, antimicrobial, NMR

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CB2	Cannabinoid receptor 2
CFU	Colony Forming Unit
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
CNS	Central Nervous System
COSY	Correlated Spectroscopy
DEPT	Distortion less Enhancement by Polarization Transfer
D-GalN	D-galactosamine
DMSO	Dimethyl sulfoxide
DPPH	1, 1-diphenyl-2-picrylhydrazyl
EALE	Ethyl acetate leaf extract
EARE	Ethyl acetate root extract
HLE	Hexane leaf extract
HRE	Hexane root extact
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
^{1}H-NMR	Proton-Nuclear Magnetic Resonance
KTER	Knowledge Transfer for Economic Research
MLE	Methanol leaves extract
MRE	Methanol root extract
PTLC	Preparative Thin layer chromatography
TM	Traditional medicine
TMS	Tetramethylsilane

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

1. INTRODUCTION

1.1.Background

Since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock. Traditional medicine refers to any ancient, culturally based healthcare practice different from scientific medicine and it is commonly regarded as indigenous, unorthodox, alternative or folk and largely orally transmitted practice used by communities with different cultures [1] .

The World Health Organization (WHO) defines traditional medicine as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses and maintain well-being [2].

The use of plants as treatment of diseases and food dates beyond recorded history perhaps as old as the history of mankind. Old civilizations in China, India, Egypt and Greece had a rich knowledge of its utility and expertise in using many types of plants. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China, and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians [3,4] .

Plants comprise the largest component of the diverse therapeutic elements of traditional health care practices both in human and animal. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines which are medicinal sources to cure infections [5].

The use of plants for medicinal purpose has a long history in many indigenous societies and they are continuing to play important roles in the treatment of various diseases, mainly in rural areas in Africa. The practices of traditional medicine are based on a long period of beliefs and empirical observations [6].

Bioactive ingredients of medicinal plants can be identified through isolation and characterization techniques. The mode of action of the plant extracts producing the therapeutic effect can also be better investigated, if the active ingredients are characterized and understood which

have led to a continuous search for potent antimicrobial agents. Natural products are believed to play vital roles in the physiology and ecology of the plants that produce them, particularly as defense elements against pests and pathogens or as attractants for beneficial organisms such as insect pollinators. Because of their biological activities, some plant natural products have been exploited by human beings as pharmaceuticals, stimulants, and poisons [7].

Much of an indigenous knowledge system, from the earliest times, is also found linked with the use of traditional medicine in different countries [8]. Even in the Western world, the use of herbal medicines is steadily growing with approximately 40% of the population reporting use of medicinal plants to treat medical illness [9, 10].

Medicinal plants based drugs owe the advantage of being simple, effective and exhibit broad spectral activity. The revival of interest in the use and importance of African medicinal plants by WHO and many developing countries has led to intensified efforts on the documentation of ethno medical data of medicinal efforts. This is because most traditional healers keep no records and their information is passed on mainly verbally from generation to generation. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections [11]

Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites [12].

Traditional remedies are part of the cultural and religious life of African people. The seemingly wide use of traditional medicine is attributed to its accessibility and affordability[13].In Africa, up to 80% of the population uses traditional medicine for primary health care. For instance, in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home [14].

In Kenya for example, 90% of the population has used medicinal plants at least once for various health conditions [15]. In Central Kenya, 75 plant species from 34 families are used to cure 59 ailments in traditional medicine [16].

In Tanzania, medicinal plants are locally used by traditional healers for the treatment of *candida* infections, including *oral candidiasis*, *vaginal candidiasis*, *oesophageal candidiasis* and skin fungal infections [17].

In Uganda, medicinal plants are used by local farmers to treat cattle (Bosindicus) ailments like East Coast Fever (ECF), abdominal worms, cataract, itching skin, wounds, measles, diarrhea and cough[18].

In West Africa, Kadiogo Province of BurkinaFaso, medicinal plants are used in the management of the following oral health concerns; acute necrotizing gingivitis, loose teeth, dental abscesses,sores in the mouth, on the tongue and lips [19]. while in Cameroon's Mbalmayo region and in the DjaBioshere Reserve and its adjacent areas in East and South provinces, medicinal plants are traditionally used in the treatment of stomach ailments believed to come from witchcraft, magic practice or vampirism, lumbago, roundworms, hernia, hookworms [20] . In Benin, medicinal plants are traditionally used to treat malaria [21].

In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also, they are integral components of the cultural beliefs and attitudes [22]. Plant remedies are still the most important and sometimes the only source of therapeutics for nearly 80% of the population [23]. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets in Ethiopia [24].

People still rely on traditional medicine system because of their effectiveness and medicinal properties that's why one fourth population of the world is following this medicine system [25,26].Therefore medicines and herbs provided by nature are used in various systems of medicine to cure health disorders [27,28,29].

Plants contain many chemical constituents which are therapeutically active or inactive. A spectrum of natural compounds like triterpenoids, alkaloids, glycosides, tannins, flavonoids, essential oils and other similar secondary metabolites which exert physiological activities are

synthesized in the plant, in addition to the carbohydrates, proteins and lipids utilized by man as food material [30].

Ocimum is widely used as a medicinal herb in the Far East, especially in China and India. It was first described in a major Chinese herbal around A.D. 1060and has since been used in China for spasms of the stomach and kidney ailments, among others. It is especially recommended for use before and after parturition topromote blood circulation. The whole herb is also used to treat snakebite and insect bites [31].

Ocimum cufodontii (Figure 1), Synonyms *O. Erythrochlamys cufodontii,* is one of the traditionally used plant species to relieve pain, fever and inflammatory disorders in Ethiopia. The plant species have distribution in Ethiopia, Somalia and Kenya. *O. cufodontii*, whose vernacular name is ‘‘Gororsisa’’, in Afan Oromo is distributed in Borana, MalcaGuba [32]. The mode of traditional administration usually involves chewing the fresh root and used as a mouth wash and astringent to cure inflammations in the mouth and throat.

However, the species *O. cufodontii* which have many traditional uses has not yet been studied for its chemical constituents and biological activities. Therefore, in this work an attempt was made to explore some biological activities of the extracts and isolated compounds from roots and leaves of *O. cufodontii*. Isolation of compounds from the leaves extract was also incorporated herein. The information spawn will be helpful in exploring the medicinal uses of *O. cufodontii*.

Figure 1: Picture of *O. cufodontii* taken by Muhdin Aliye in East Arsi Zone Merti woreda, Besicho kebele, on Oct 15, 2017.

1.2. Statement of the problem

There has been an increase in diseases and conditions such as diabetes, high blood pressure, heart diseases, inflammation, bacterial, bronchitis and cancers. One of the major remedies to partly alleviate this problem in developing countries is the use traditional medicines. Given the increased use of traditional medicines, possibilities that would ensure their successful integration into a public health framework should be explored. In recent years, secondary plant metabolites, previously with unknown pharmacological activities, have been investigated as a source of medicinal agents [33]. These phytochemicals include alkaloids, saponins, tannins, flavonoids, steroids, cardiac glycosides among others. A lot of work has been reported on the organic composition of the traditional medicine [34].

Demissew and Asfaw (1994) [35] reported on *O.obovatum, O. suave* and *O. lamiifolium,* medicinal plants widely used in Ethiopia against various diseases. However, to the best of my knowledge little scientific reports on the chemical constituents and pharmacological activities of their sister species, *O. cufodontii,* is documented in the literature. Therefore, this thesis explores some biological activities of the extracts and isolated compounds from roots and leaves of *O. cufodontii*.

1.3. Significance of the study

The information obtained on the assessment of this plant species will help in documentation of the medicinal plant and ailments they used treat. The work may also help to know chemical composition of the leaves and roots of *O.cufodontii*. The results of this study may help in the formulation of drug from natural products. The information gathered may be used to increase awareness of people on the role of the traditional uses of *O.cufodontii*. The finding will also be used as a baseline study for other researchers working on medicinal plants.

1.4. Objectives of the study

1.4.1. General objective of the study

The overall objective of this work is to conduct phytochemical investigation and some biological activities of the roots and leaves extracts of *O. cufodontii* .

1.4.2. Specific objectives

The specific objectives of this study are to

- successively extract the roots and leaves of *O. cufodontii* using n-hexane, ethyl acetate and methanol.
- extract the essential oil of the leaves of *O. cufodontii* using hydro-distillation
- analyze the essential oil of the leaves of *O. cufodontii* using GC-MS
- conduct phytochemical screening of the crude extracts.
- isolate compounds from the extracts of the roots and leaves of *O. cufodontii* using column chromatography and PTLC.
- characterize structures of the isolated compounds using different spectroscopic techniques including UV, IR and NMR.
- evaluate the antibacterial activities of the extracts and isolated compounds using cup diffusion method against (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonae* ,*Salmonellatyphi* and *Staphylococcus aureus*).
- evaluate the antibacterial activities of the essential oil of the leaves of *O. cufodontii* using cup diffusion method against *Staphylococcus aureus* .
- assess antioxidant activities of the extracts and isolated compounds using DPPH radical scavenging assay.

2.0. LITERATURE REVIEW

2.1. The family Lamiaceae

The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the volatile oils concentration [36]. The Lamiaceae plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil [37]. This oil is important in pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries[38].

The family Lamiaceae comprises of 258 genera and 6,970 species. The plant grows in the form of herbs, shrubs or trees having opposite or whorled leaves. This plant has highly economic importance and was traditionally used in medicines [39].The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many of the family, particularly subfamily Nepetoideae, to which *Ocimum* belongs, are strongly aromatic due to essential oils which consist of monoterpenes, sesquiterpenes and phenylpropanoids.

2.2. The Genus *Ocimum*

The *Ocimum* genus consists of 65 species possessing dramatically different chemical compositions[40]and is comprised of 200 species differentiated from each other on the basis of morphological features and chemical constituents[41].

Ocimum includes annual and perennial herbs, as well as shrubs endemic to the tropical and subtropical regions of Asia, Africa and Central South America [42]. *Ocimum sanctum, Ocimum gratissimum* (Ram tulsi), *Ocimum canum* (dulal tulsi), *Ocimum basilicum* (Ban tulsi),*Ocimum killimandscharicum*, *Ocimum americanum, Ocimum camphora* and *Ocimum miranthum* are examples of known important species of genus *Ocimum* that grow in the different parts of world and are known to have medicinal properties [43-45].

2.3. Biological activities of the genus *Ocimum*

Ocimum has been extensively used in Ayurvedic system of medicine for various ailments including capability of lowering plasma glucose, antibacterial [46] , antimicrobial [47] , hyperlipidemic, insecticidal, CNS stimulant, memory enhancer, in treatment of bronchitis, cough etc. *Ocimum* has gained a great interest in scientific research because of the presence of natural flavor like essential oils and herbal extracts. These naturally occurring mixtures having chemical composition especially the essential oils must be safely kept according to scientifically based guide because they were commonly used as flavoring agents [48].

2.4. Botanical descriptions of *Ocimum cufodontii*

According to Flora of Tropical East Africa, [49] *O. cufodontii* is an aromatic perennial shrub with a height to1 m tall. Stems rounded-quadrangular, woody, branched; indumentum tomentose, hairs short, dense and dendroid. Leaves with or without young shoots in axils; blades ovate to broadly ovate, 1.5–6 × 0.8–3.5 cm, serrate or sub entire, apex obtuse, base cuneate, with short dendroid hairs on both surfaces; petiole 3–15 mm.

Calyx horizontal or downward-pointing, 3 mm at anthesis, indumentum brownish, tomentose, longer on tube and anterior lip than on posterior lip; posterior lip large, rounded at tip, decurrent, tomentose on inner surface; four lobes of anterior lip subequal, bristle-pointed; fruiting calyx 7–10 mm long, throat closed, posterior lip expanded, rotund, decurrent, obscuring the rest of the calyx from above, accrescent or flat, median lobes of anterior lip pressed against underside of the posterior lip, teeth of lateral lobes lower than or level with the underside of the median lobes, often spreading.

Corolla greenishwhite, white or yellow, 6–7 mm long, tube straight, funnel-shaped, scarcely exceeding calyx,lips tomentose. Stamens exceeding corolla by 2–3 mm, posterior with a large, fleshy, flattened, hairy outgrowth near base. Fruits are Nutlets brown, spherical, 1 mm in diameter, pubescent, not producing mucilage when wet and finally the ecology were *Acacia-Commiphora* bushland on lava and limestone, *Acacia* bushland; 350–1200 m.

Antibacterial Activity

Aqueous extracts of *O.gratissimum* showed no activity against the test organisms (*Salmonella spp., Shigella sonnei, Shigella schmitzi, Staphylococcus aureusand Escherichiacoli).*This indicates that the antibacterial principles are not water soluble. A high phenol content, in this case thymol, of the oil gives a higher antibacterial activity [50, 51, 52]. *O. basilicum* shows antibacterial activity [53]. *O. micranthum* was reported as to have antibacterial, antiprotozoal and antioxidant activity [54] while *O. americanum* have antibacterial effect [55].

Anticarcinogenic Effects

An ethanolic extract of the leaves of *O.sanctum* showed chemopreventive effects on 7,12-dimethylbenzanthracene induced skin papilloma genesis in male Swiss albino mice. A significant reduction in the tumor incidence, average number of tumors/ tumor bearing mice and the cumulative number of papillomas was observed in mice treated topically with the leaf extract of *O.sanctum* [56]. *O.basilicum* helps in the treatment of colon tumors [57] and also shows anticancer activity [58].

Hypoglycemic and Blood Lipid Lowering Effect

One of the traditional uses for *O.sanctum* is for treating diabetes. Experimental studies performed in the 1960's showed that basil leaves can decrease blood glucose in hyperglycemic rats. These results were confirmed kter by other studies which showed that oral administration of alcoholic extracts of *O.sanctum* leaves led to a marked lowering of blood sugar level in normal, glucose fed hyperglycemic and streptozotocin induced diabetic rats [59, 60].

Fresh leaves of *O.sanctum* (1 and 2 g/100 g diet) given for 4 weeks, brought about significant changes in the blood lipid profile of normal albino rats. Serum total cholesterol, triglycerides, phospholipids and LDL cholesterol decreased and HDL cholesterol and total faecal sterol content increased [61].*O. basilicum* shows decreased cholesterol synthesis and *O. sanctum* shows hypoglycemic [62] and anti lipidemic activity [63]. *O. americanum*(syn. *O. canum*) also shows hypoglycemic activity [64].

Effects on the Central Nervous System

An ethanolic extract of the leaves of *O.sanctum* was screened for its effects on the central nervous system. It prolonged the sleeping time induced by pentobarbital, reduced the recovery time and severity of electroshock and pentylenetetrazole-induced convulsions and decreased the apomorphine-induced fighting response and open-field activity. All these actions resemble the activity of low doses of barbiturates. The mechanism of action for the *Ocimum* extract may involve dopaminergic neurones [65] and also shows neuroprotective effect [66]. *O. gratissimum* also used in treatment of CNS disorders [67].

Antiulcerogenic Effect

Aqueous and methanolic extracts of *O.basilicum* showed antiulcerogenic effects when administered to rats with aspirin-induced gastric ulcers. The ulcer index was decreased by both extracts. The acid output was decreased only by the methanolic extract, which indicates that the active principles are soluble in methanol. The pepsin output was decreased by both the aqueous and methanolic extract in ulcerated, but not in normal animals. The aqueous extract of *O.basilicum* also increased the hexosamine concentration in normal rats, which might contribute to its antiulcer effect [68, 69].

The antiulcerogenic effects of extracts, volatile oils and flavonoid glycosides of *O. basilicum* leaves were studied in normal as well as aspirin-acetic acid- and stress-induced ulcerated rats. Their effect on the output of gastric acid, pepsin and hexosamines was recorded. The aqueous, methanol and water-methanol extracts and flavonoid glycosides decreased the ulcer index, inhibited gastric acid and pepsin secretion and enhanced hexosamines. It appears that the antiulcerogenic compounds of *O.basilicum* are extractable both into water and methanol and that they may include flavonoid glycosides. These substances may act by augmenting the gastric barrier [70].A study on *O.sanctum* showed that the extract reduced the ulcer index, free and total acidity on acute and chronic administration. It also increased the mucous secretion [71].*O. sanctum* also shows antiulcer activity [72].

Hepatoprotective Activity

O.gratissimum and *O.basilicum* are used in a Taiwanese herbal remedy believed to possess anti-inflammatory and detoxication activities. The crude extracts were studied against $CC1_4$ and D-GalN-induced acute hepatitis and were found to be hepatoprotective [73].

Insecticidal Activity

O. basilicum shows insecticidal activity [74] and *O. americanum* is insect repellent [75]. The essential oil (2% in acetone) of *O.gratissimum* showed 100% repellency against the housefly, *Musca domestica* [76]. *O. suave* essential oil was found to repel and kill all stages of the tick *Rhipicephalus appendiculatus*.

2.5. Phytochemistry of genus *Ocimum*

O. gratissimum leaves contains clocimum oil having eugenol as methyl eugenol or eugenol acetate**(1),** cis-ocimene **(2)** , germacrene-D **(3)** , β – caryophyllene **(4)**, β-pinene **(5)** [77]. *O. cannum* leaves contain camphor **(6),** limonene (**7**), β-caryophyllene (**4**), Camphene (**8**) methyl Chavicol (estragole) (**9**) [78]. *O. kilimandscharicum* leaves and flowers contains linalool (**10**), camphor (**6**), 1, 8-cineole (**11**) and Camphene (**8**) [79].

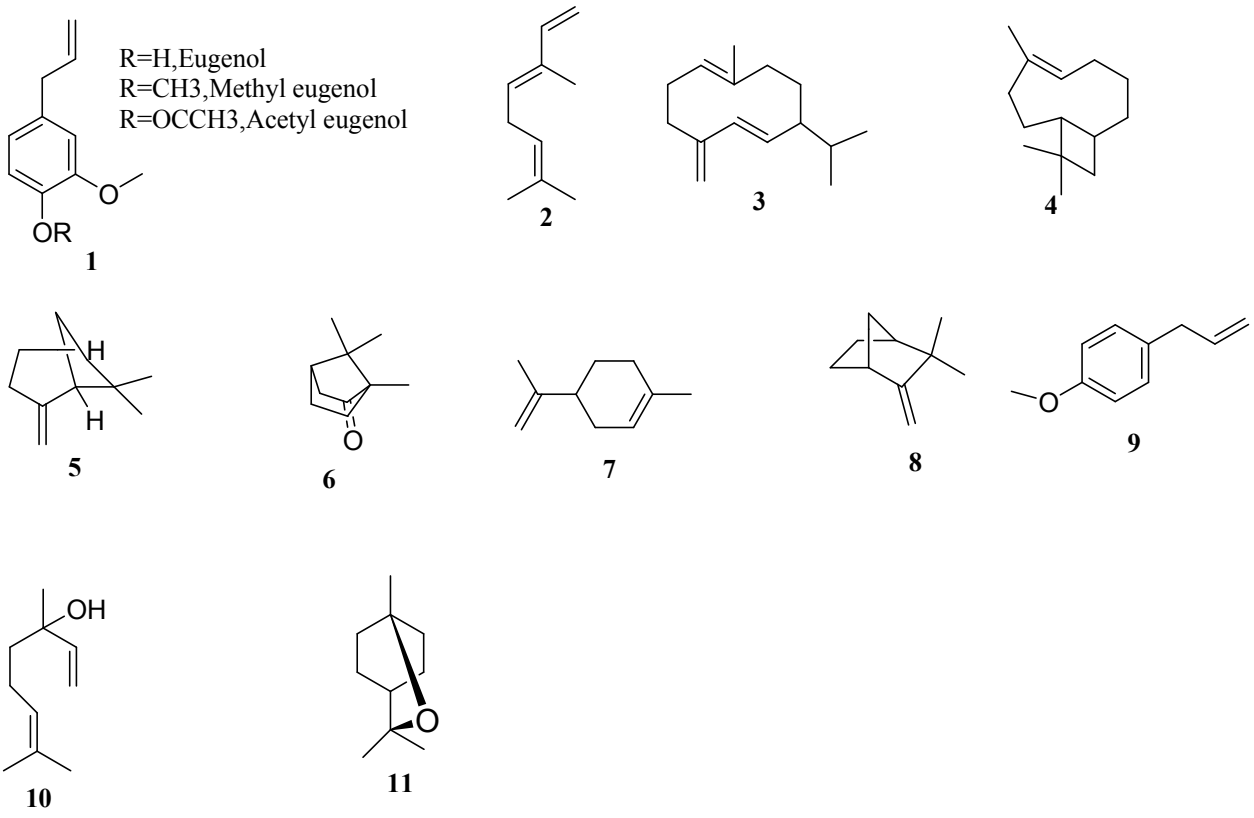
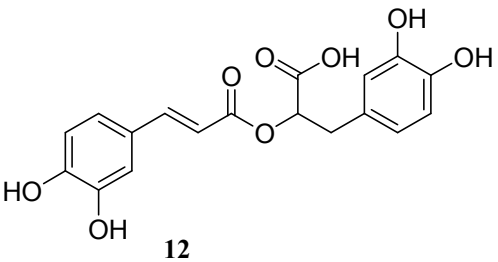
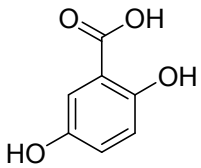


Figure 2: Chemical constituents from the oils of the leaves and flowers of *O. gratissimum* and *O. kilimandscharicum*

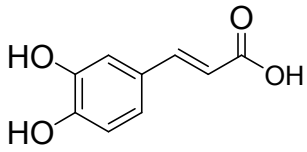
Tarchoune *et al*., 2009 [80] also found rosmarinic (**12**), gentisic (**13**) and caffeic acids (**14**) as main phenolic acids of fresh leaves of 'Fine' and 'Genovese' varieties of *O. basilicum* from Tunisia extracted with chloroform/methanol .



12



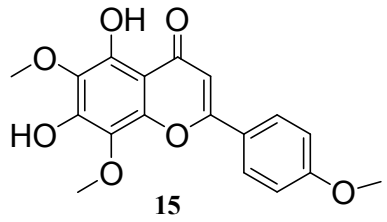
13



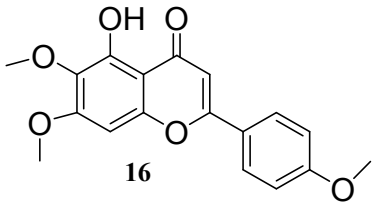
14

Figure 3: Phenolic acids reported from leaves of *O. basilicum*

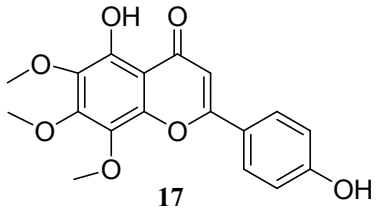
Navadensin(**15**) and salvigenin(**16**) from *O. canum* and xanthomicrol(**17**), eriodictyol (**18**), eriodictyol-7 glucoside (**19**) and vicenin-2 (**20**) from *O. basilicum* have been isolated [40]. Rosmarinic acid (**12**), gallic acid (**21**), vanilic acid (**22**), protocatechuic acid (**23**), 4-hydoxybenzoic acid (**24**), Vanillin (**25**), Cafeic acid (**14**) and Chlorogenic acid (**26**) were isolated phenolic acids from leaves of *O.sanctum* [40]. Ursolic acid (**27**) and oleanolic acids (**28**) as tri terpenes were also reported in *O. sanctum* [81].Also, [82] detected rosmarinic acid (**12**) as a major phenolic acid in the local accessions of *O. basilicum* grown in Iran. *O.basilicum* aerial parts contains linalool (**10**),(Z) cinnamicacidmethylester(**29**), cyclohexene(**30**),α-cadinol(**31**),2,4-diisopropenyl-1-methyl-1-vinylcyclohexane(**32**),3,5-pyridine-dicarboxylic acid(**33**), cubebene (**34**), guaia-1(10) 11-diene(**35)**, cadinene (**36)**, (E)- Cinnamic acid methyl ester (**37**) , β-guaiene (**38**) [83].



15



16



17

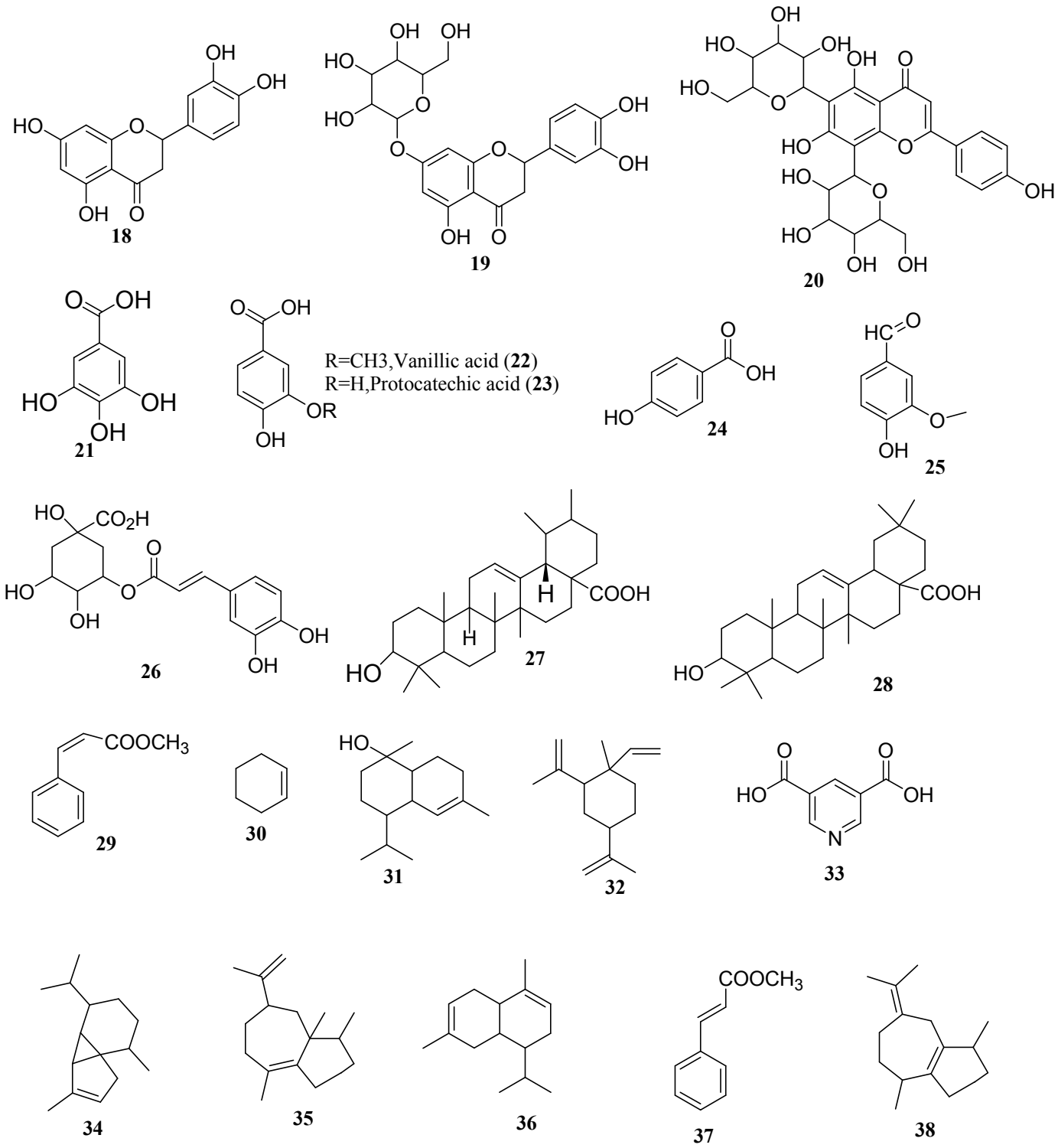


Figure 4: Some of the isolated phenolic acids and triterpenes from *O. canum, O. basilicum* and *O.sanctum*

3.0. MATERIALS AND METHODS

3.1. Reagent and chemical used

The chemicals that were used in this study include [n-hexane (99%), ethyl acetate(99.5), methanol (99.8%,HPLC grade), chloroform(99%)] (LOBA), concentrated HCl (37% ,Carloerba), saturated solution of sodium hydroxide (LOBA), sodium carbonate, ethanol, distilled water, DPPH and silicagel (LOBA).

3.2. Apparatus and instruments

Column chromatography was performed on normal silica gel (mesh size 200-400). Thin layer chromatography was done using silica gel $60F_{254}$ (Merck) precoated plates. Its melting point was determined using Melting point apparatusM-565 (BUTHI, Germany). NMR analyses were carried out on Bruker Avance 400 MHz spectrometers. Structural assignment will be performed based on COSY, HSQC, and HMBC. DPPH scavenging activity has been done using a JETWAY6300 spectrophotometer.

GC-MS has been carried out using GC-MS Agilent technologies. A GC-MS instrument from Agilent Technologies (Santa Clara, CA, USA) equipped with a 6890N network GC system, 5975 inert mass selective detector, 7683B series auto sampler injector (10 µl in size), G1701DA GC/MSD Chem Station and HP5MS column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) coated with 5% phenyl 95% methyl poly siloxane was used for analyzing the samples. 2 µl essential oil solutions in chloroform was injected through auto sampler and analyzed with HP5MS column. Column temperature was programed as follows: 55°C to 120°C at 20°C/min, 120°C to 150°C at 1.5°C/min, 150°C to 250°C at 20°C/min, 250°C (10 min) and 3 min solvent delay. The mass spectra transfer line temperature was 280°C. The carrier gas was helium (1 ml/min) with a split ratio equal to 100:1. Injector, quadruple and detector temperatures were 220, 150, and 250°C, respectively. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 50 to 500 amu (atomic mass unit) at 0.5 sec with the mass source being set at 230°C. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Interpretation of mass spectrum of GC-MS can be compared their mass spectra with the NIST 2005 library of mass spectra. Integration of peaks was performed using GC/MSD Chem Station software for quantification of the peaks.

3.3. Plant material collection and identification

The root and leaves of *O. cufodontii* were collected on October 15, 2017from Besicho kebele (which is 92 km East from Adama), Arsi Zone Merti Woreda, Oromia region, Ethiopia. This Woreda is situated at an altitude of 1438 meters above sea level. The annual temperature ranges from 8°C to 39°C [84].The plant was identified by Mr. Shambel Alemu and stored in the National Herbarium of Addis Ababa University with voucher specimen MA001.

3.4. Experimental procedures

3.4.1. Extraction

The collected plant material was air dried and grinded to a uniform size using an electric grinder. Ground roots of *O. cufodontii* (300 g) were successively extracted with each 1.5 L of *n*-hexane, EtOAc and MeOH for 72 hrs on maceration. Each extracts was filtered and concentrated using rotary evaporator to furnish their corresponding extracts. The extracts were analyzed with TLC. Likewise, the leaves were also extracted following same procedure as above.

3.4.2. Isolation

Each extracts were pre-analyzed with TLC. The isolation and purification of compounds have been carried out over silica gel column chromatography. The elution progress of each fraction and sub fractions were monitored by TLC. Further chromatographic analysis like PTLC has been carried out for further purification. Visualization of the chromatogram was achieved by UV lamp and spraying with vanillin as spray reagent.

The EtOAc extract (5 g) of the leaves of *Ocimum cufodontii* was pre-adsorbed and subjected to silica gel (150 g) column chromatography with n-hexane: EtOAc of increasing polarities as eluent to furnish 58 fractions, each 50 mL. Fraction 24 collected with hexane: EtOAc (7:3) gave 40 mg of compound **39**.

The hexane extract (3.0 g) of the roots of *Ocimum cufodontii* was pre-adsorbed and introduced on silica gel (150 g) column chromatography with increasing polarities EtOAc in n- hexane as eluent to give 24 fractions, each 50 mL. Fraction 4 eluted with hexane: EtOAc (85:15) furnished 32 mg of compound **40** as yellow crystals. Fraction 10 eluted with hexane: EtOAc (4:1) was found as a red crystal to furnish 38mg and labeled as compound **41**.

The EtOAc extract (3.0 g) of the roots of *Ocimum cufodontii* was pre-adsorbed and introduced on silica gel (150 g) column chromatography with increasing polarities EtOAc in n- hexane as eluent to give 41 fractions, each 50 mL. Fraction 7 eluted with hexane: ETOAc (9:1) afford 30 mg of compound **42**. On the other hand fraction 37 and 38 eluted with hexane: EtOAc (3:7) were merged together to furnish compound **43** as a yellow solid.

3.4.3. Extraction of essential oil by hydro-distillation

The ground leaves of *Ocimum cufodontii* (60 g) were placed in 1000 mL round bottom flask. Then 600 mL of distilled water was added and mixed thoroughly. The flask was fitted with Clevenger's apparatus, a glass condenser and heated using heating mantle and hydro distilled at atmospheric pressure for 4 hrs. The oil was separated from the aqueous layer by adding 100 mL of chloroform in a separatory funnel, dried over anhydrous sodium sulfate, filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator. This was kept in the refrigerator until analyzed with GC-MS.

Essential oil yield was calculated on dry weight basis by using following formula.

$$EO\% = \frac{\text{Volume of essential oil (mL)}}{\text{Weight of dried material}} \times 100\%$$

3.5. Qualitative analysis of the chemical constituents of crude extracts

The n-hexane, ethyl acetate, methanol root extracts and the leaves powder was subjected to qualitative chemical analysis. The tests was done to detect the presence of secondary metabolites such as alkaloids, glycosides, terpenoids and steroids, flavonoids ,reducing sugar and tannin by the following procedure.

3.5.1. Test for alkaloids

- **Wagner's Tests (**Solution of Iodine in Potassium Iodide**)**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The acid layer with few drops of Wagner's reagent would give reddish brown colored precipitate.

3.5.2. Test for glycosides

Cardiac glycoside

- **Keller-Killani Test:** To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulfuric acid were added in a test tube. Reddish brown color appeared at the junction of the two liquid layers was the indication for the presence of cardiac glycosides.

Anthraquinone glycosides

- **Borntrager's Test:** To 3 ml extract dilute sulfuric acid was added, boiled and filtered. To the cold filtrate equal volume chloroform was added. The organic layer was separated and ammonia was added. Ammonical layer turned to pink or red was the indication of Anthraquinone glycosides.

Saponin glycosides

- **Froth Test:** In 20 ml distilled water the extracts were diluted and shaken in a graduated cylinder for 15 minutes and 1cm layer formed was conformation for the presence of saponin.

Coumarin glycosides

- Alcoholic extract when made alkaline, showed blue or green fluorescence.

3.5.3. Test for terpenoids and steroids

- **Salkowaski Test :** Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids [85].

3.5.4. Test for flavonoids

- **Shinoda Test:** Extracts were treated with few drops of sodium hydroxide solution and Formation of intense yellow color, which becomes colorless on addition of dilute acid, was the indication of flavonoids [86].

3.5.5. Test for reducing sugar

- **Fehling's Test:** 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

3.5.6. Test for Tannins

- **Ferric chloride test:** 0.5 ml of extract solution, 1 ml of water and 1-2 drops of 1 % ferric chloride solution was added in a test tube. Blue color would be observed for gallic tannins and green black for catecholic tannins [87].

3.5.7. Test for phytosterols

- **Liebermann Burchard test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulfuric acid would be added and brown ring at the junction indicated the presence of phytosterols.

3.5.8. Test for Phenolic Compounds

- **Ferric chloride Test:** 2 g of the extract of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1 % $FeCl_3$and 1 ml of potassium ferrocyanide were added to detect phenolic compounds. Bluish green color indicated the presence of phenolic compounds [86].

3.6. Antibacterial assay

3.6.1. Antibacterial activity

Antibacterial activity of the different extracts was determined by cup diffusion method on nutrient agar medium [88]. Wells have been made in nutrient agar plate using cork borer (5 mm diameter) and inoculums containing 10^6CFU/ml of bacteria have spread on the solid plates with a sterile swab moistened with the bacterial suspension and fifty micro-liters of the working suspension/solution of different medicinal plant extract and same volume of extraction solvent for control will be filled in the wells with the help of micropipette. Plates have been left for some time till the extract diffuse in the medium with the lid closed and incubated at 37° C for 24 h. After overnight incubation the plates was observed for the zone of inhibition (ZI) and the diameter of the inhibition zone will be measured using scale and mean will be recorded. The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotics: chloramphenicol (30 mg) for bacteria. chloramphenicol was used as positive control and the solvent DMSO as negative control. An inhibition zone of 10 mm or greater was considered to indicate good antibacterial activities.

3.7. Antioxidant Activity

3.7.1. DPPH-free radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al*. (2001) [89].The hexane extract of the leaves of *O. c* was dissolved in methanol and serially diluted with the same amount of 0.004% methanol solution of DPPH to furnish 200, 100, 50, 25 and 12.5 µg/mL.

The resulting solutions were incubated in the dark at room temperature for 30 minutes and the absorbance at 517nm was determined using UV-Vis spectrometer (JENWAY6300). The percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard.

The radical scavenging activity will be measured as the decrease in absorbance due to DPPH radical expressed as a percentage of the control solution (consisting of 2 ml of methanol plus 3 ml of 0.004% DPPH solution). Ascorbic acid was chosen as the reference antioxidant for this test.

The activity will then be expressed as IC_{50}, which is the concentration of the test compound required to give a 50% decrease of the absorbance from that of the control solution. The inhibition curves were prepared and IC_{50} values were obtained.

The radical scavenging activity of the EtOAc extract of the leaves, the hexane and EtOAc extract of the roots of *Ocimum cufodontii* was also done following similar procedure.

4.0. RESULTS AND DISCUSSION

4.1. Extract Yield

The leaves of *Ocimum cufodontii* was extracted with EtOAc and MeOH to furnish 2% and 1.5 %, respectively. Likewise, the root was extracted with hexane and EtOAc to give 1.5% and 1.2 %, respectively. As revealed above the secondary metabolites obtained from the roots and leaves are mainly non-polar and medium polar respectively. On the other hand, the hydro-distillation of the air dried ground leaves of the plant yielded pale yellowish essential oil of (16.66% w/w).

4.2. TLC analysis of the extracts

Each extracts from the leaves and roots of *Ocimum cufodontii* were analyzed with TLC with the results presented in Figure 5. As seen from the TLC profile above, the hexane extract of the root and the EtOAc extract of the leaves were shown to have good spots for further fractionation.

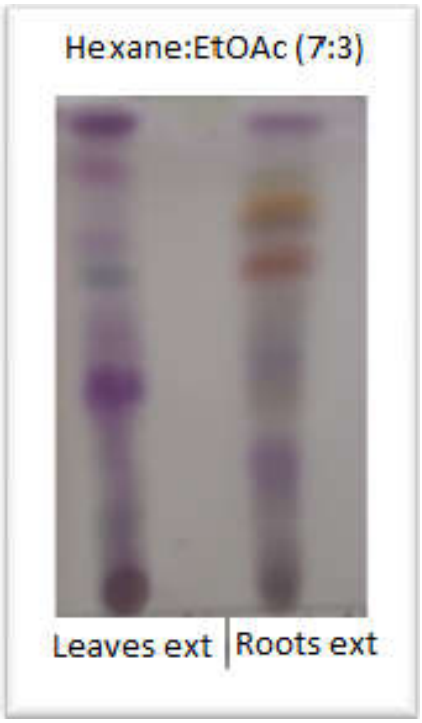


Figure 5: TLC profile of the crude extracts

4.3. Phytochemical Screening

The hexane, EtOAc and MeOH extract of the leaves and roots of *Ocimum cufodontii* were analyzed for the presence of secondary metabolites including alkaloid, tannins, steroids, terpenoids, phenols, flavonoids, anthraquinone glycosides,saponin glycosides, cardiac glycosides and reducing sugars following standard procedure [85]. The results obtained were summarized in Table 1.

Table 1: Phytochemical screening in hexane, ethylacetate and methanol leaf and root extract of *Ocimum cufodontii*

		Hexane extracts		Ethylacetate extracts		Methanol extracts	
Phytochemicals	Test	Leaf	Root	Leaf	Root	Leaf	Root
Alkaloids	Wagners	+	+	+	+	-	+
Saponin glycosides	froth	+	+	_	+	_	+
Tannins	Ferric chloride	_	_	+	_	_	_
Anthraquinones Glycosides	Borntrager's	_	+	_	+	_	_
Steroids	Salkowiski	_	+	+	+	_	_
Terpenoids	Salkowiski	+	+	+	+	+	+
Flavonoids	Shinoda	_	+	_	+	_	+
Cardiacglycosides	Keller-killiani	+	_	_	+	_	+
Coumarin glycosides	Keller-killiani					_	+

Reducing sugar	Fehling's solution	+	+	_	+	_	_
Phytosterol	Lieberman buchard	_	+	_	_	_	+
Phenols	Ferric chloride	_	_	+	_	+	+

+ = Present

- = Absent

In this study, the plant was screened for the presence of secondary metabolites. Results from this present study showed phytochemicals ranging from tannins, alkaloids, flavonoids, saponins, terpenoids to sterols were present.

4.4. Characterization of isolated compounds

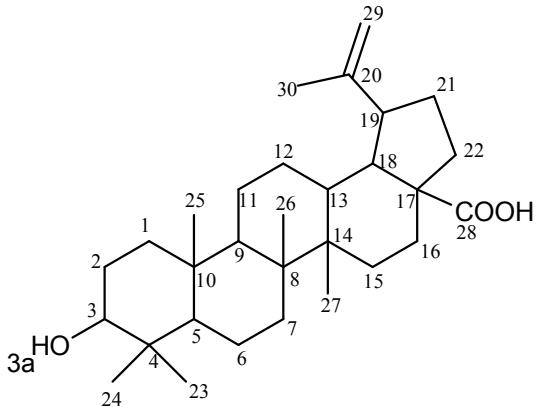
In the course of this work, one compound from the leaves and four compounds from the roots were isolated. The characterizations of these compounds are described herein.

Compound **39** was isolated as a white solid with melting at 316-318°C. The pink spot on TLC plate visualized on spraying with vanillin showed its terpenoidal nature; it has an Rf value of 0.47 in hexane: ethyl acetate (7:3) which was similar to that reported for betulinic acid by [90, 91, 92].

Compound 39

The mass spectrum of Betulinic acid **(39)** displayed a molecular ion peak $(M-H)^+$ at m/z 455 corresponding to the formula $C_{30}H_{48}O_3$.The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum showed signals in the paraffinic and olefinic region. Signals attributable to an exo-methylene group at δ 4.64/4.51 (2H, *d*, *J* =2.0, H-29) which together with an allylic methyl at δ1.61 (3H, *s*, Me-30) which indicated an isopropenyl function. The ^{1} H NMR spectrum showed the signals for five tertiary methyl groups at δ 1.18, 0.74, 0.67, 0.87 and 0.67, one isopropenyl moiety at δ 1.61, 4.51 and 4.64, indicating a lupane-type skeleton .The up-field signals at δ 1.18 (3H, *s* ,H-23) and δ 0.74 (3H, *s* , H-24) were due to methyl protons at C- 23 and C-24, respectively. In

addition, the spectrum also showed a multiplet at δ 2.93 (1H, *m*, H-19) and δ 1.50 (1H, *m* , H-18) for the methine protons at position 18 and 19, respectively.



39

Figure 6: Structure of betulinic acid **(39)** isolated from the leaves of *Ocimum cufodontii*.

These spectral features are in close agreement to those documented and reported by [90, 91, 92] (Table 2) for betulinic acid.

Table 2: ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR (100 MHz) data of compound **39** and literature reported for betulinic acid.

No.	^{1}H-NMR data of compound-39 (δ)	^{13}C-NMR data of compound-39 (δ)	Lit. reported for betullinic acid [91]	Lit. reported for betullinic acid [93]
1	1.86 (2H, *m* , H-1)	38.7		39.1
2	1.61 (2H, *m*, H-2)	27.7		28.1
3	3.29 (1H,*d*, *J* =3.41,H-3) 3.11 (1H, *d*, J= 8.0Hz ,H-3a)	78.7	3.18(1H, *dd*, *J*=11.2,4.9 Hz,H-3)	78.1
4	-	38.7		39.4
5	1.35 (1H ,*m*, H-5)	56.1		55.7
6	1.42 (2H, *m* , H-6)	18.2		18.6
7	1.43 (2H, *m,* H-7)	34.2		34.7

8	-	40.6		40.9
9	1.68 (1H, *m* , H-9)	50.4		50.8
10	-	37.0		37.3
11	1.69 (2H, *m* , H-11)	20.8		21.1
12	1.29 (2H, *m*, H-12)	25.4		25.9
13	1.43 (1H, *m* , H-13)	38.2		38.4
14	-	42.3		42.4
15	1.53 (2H, *m* , H-15)	30.5		31.1
16	1.57 (2H, *m*, H-16)	32.1		32.7
17	-	56.1		56.3
18	1.50 (1H, *m* , H-18)	46.9		47.6
19	2.93 (1H, *m*, H-19)	49.2	2.98 (1H, *m*, H-19)	49.5
20	-	150.6		150.7
21	1.86 (2H ,*m* , *J* = 4.0, H-21)	30.5		30.1
22	1.84 (2H,*m*, H-22)	37.1		37.5
23	1.18 (3H, *s* ,H-23)	27.7	0.96 (3H, *s* ,Me-23)	28.5
24	0.74 (3H, *s* , H-24)	15.9	0.74(3H, *s*, Me-24)	16.3
25	0.67 (3H , *s* , H-25)	15.7	0.81 (3H, *s*, Me-25)	16.3
26	0.87 (3H, *s* , H-26)	15.7	0.97 (3H, *s* , Me-26)	16.2
27	0.67 (3H , *s* , H-27)	14.5	0.93 (3H, *s* , Me-27)	14.8
28	-	179.1		178.7
29	4.64/4.51 (2H, *d* , *J* = 2.0, H-29)	109.3	4.73 (1H, *d* , *J* = 2.34Hz,Ha-29) 4.60(1H,d,*J*=2.02 Hz, Hb-29)	110.3

30	1.61 (3H , *s* , H-30)	19.2	1.68 (3H,*s*,Me-30)	19.4

The ^{13}C-NMR spectrum revealed the presence of 30 carbon signals Table 2 which were shown to be six methyl, six quaternary carbons, one carboxylic acid, eleven methylene of which one are due to olefinic carbons and six methine suggesting that compound **39** is a triterpenic acid having five rings [93]. Compound **39** was identified as betulinic acid (Figure 6) by direct comparison of its spectral data with reported values in Table 2.

Betulinic acid is a common secondary metabolite of plants found in the Betula species (Betulaceae). It was also isolated from species such as *Ziziphus mauritiana*Lam (Rhamnaceae) collected in Zimbabwe [94] and as reported from [95] leaves of *Ocimum canum,* yield betulinic acid. This appears to be the first report of the presence of betulinic acid from sister species *Ocimum cufodontii*.

Compound 40

Compound **40** was isolated as light yellow solid (32 mg) from n-hexane root extract which yields 0.28%. The UV-VIS (MeOH) showed absorption maxima at 140, 240 and 290 nm suggesting an anthraquinone skeleton [96].

The ^{1}H NMR spectral data (Table 3) revealed that ring A of the quinone skeleton is di substituted at C-2 and C-3 positions with methoxyl δ 3.90 ppm and ring D is di substituted at C-14 and C-15 with methyl (δ 0.96 and δ 0.97 ppm) respectively. In agreement with this, the ^{I3}C NMR spectral (Table 3) revealed the presence of twenty-two carbons: two carbonyls (δ 189.7 and δ 184.2), two oxygenated quaternary carbons at δ 157.1 and δ 150.7 , five methine carbon (*δ* 21.7 , *δ* 35.8, *δ* 41.1, 33.2 and *δ* 24.3), three non-oxygenated quaternary carbons at *δ* 140.4 ,135.6 and 33.0. The two methoxy carbon *δ* 60.7 and *δ* 62.7 attached were observed. Besides, the three methyl group at *δ* 20.5, 18.6 and 18.8 were revealed from ^{I3}C NMR spectral. Finally five methylene carbons at *δ* 84.4, *δ* 20.3, *δ* 39.3, 26.0 and *δ* 45.9 were revealed from DEPT spectra.

Table 3: ^{1}H NMR and ^{13}C NMR spectral data of compound **40**

No.	^{1}H-NMR data of compound 40 *(δ)*	^{13}C-NMR data of compound 40 *(δ)*
1	-	189.7
2	-	157.1
3	-	150.7
4	-	184.2
1a	-	140.4
4a	-	135.6
5	3.18(1H,*m*)	21.7
6	1.94(1H,*m*)	35.8
7	4.75(2H,*d*,*J*=1.2HZ)	84.4
8	1.54(2H,*m*)	20.3
9	1.47(2H,*m*)	39.3
10	-	33.0
11	1.28(3H,*m*)	20.5
12	1.52(1H,*m*)	41.1
13	1.47(2H,*m*)	26.0
14	1.67 (1H,*m*)	33.2
15	1.63 (1H,*m*)	24.3
14-a	0.96(3H,*m*)	18.6
15-a	0.96(3H,*m*)	18.8
16	1.32(2H,*m*)	45.9
CH_3O-	3.90 (3H, *s*)	62.7

CH_3O-	3.90(3H,*s)*	60.7

The spectral data of compound **40** agreed with 8,9-Dimethoxy-2,3,12a-trimethyl-2,3,4,4a,4b,5,10b,11,12,12a-decahydro-1H-6-oxa-chrysene,7,10-dione (**40**) whose structure is given in Figure 7.

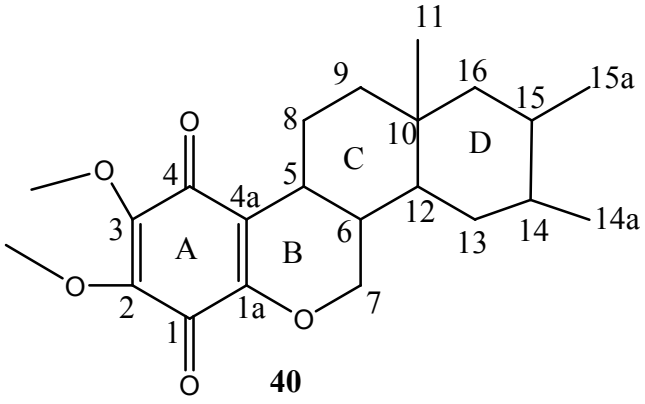


Figure 7: The structure of compound **40** isolated from the roots of *Ocimum cufodontii*

Compound 41

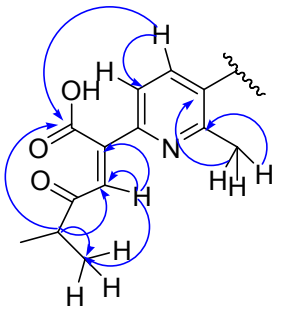
Compound **41** was isolated as a red crystal (32 mg) from the hexane extract of the root of *O.cufodontii* which melted at 85^0C. The ES-MS spectrum revealed the molecular ion peak m/z 330.2 $[M-H]^+$ attributed to the molecular formula $C_{19}H_{25}O_4N$. The UV-VIS (Methanol) showed absorption maxima at 340 and 540 nm suggests the presence of conjugated chromophore in the structure.

In the ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) signals due to four methyl groups were evident at δ 1.12 (6H, *d*, *J* = 6.94 Hz, H-7'',8') and δ 1.08 (6H, *d*, *J* = 6.77 Hz, H- 6',7'). Besides the proton at the multiplicity observed indicated that the two methyl groups are directly attached to methine carbon. This is confirmed from the observed signals due to the two methine group at δ 2.32 (3H, *s*, H-6a) and δ 2.63 (2H, *t*, H- 4''). The three olefinic proton at δ 7.07 (1H, *s*, H-3') , δ 7. 04(1H, *d, J* = 8.42 Hz, H-3) and δ 7.35 (1H, *d*, *J* = 8.42 Hz, H-4) were confirmed from the ^{1}H NMR. The proton at δ 7.35 and δ 7.07 were correlated with each other in the COSY spectrum indicating that the two protons are adjacent to each other. The signals due to the two methylene protons-were observed at δ 3.12 (2H, *t*, *J* = 6.84, H-3'') and δ 2.67 (2H, *t*, *J* = 6.84, H-4'') which are located in between the free ketone and aromatic ring were confirmed from ^{1}H NMR. This is confirmed from the COSY and HMBC correlation.

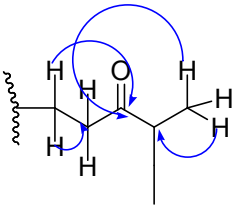
The ^{13}C NMR spectral (Table 4) revealed the presence of three carbonyl carbon at carbon position C-5'', C-1' and C-4' with their signal observed at δ 213.6, δ 181.2 and δ 182.3 respectively. The ^{13}C NMR with the aid of DEPT-135 revealed the presence of two methylene carbons at δ 24.9 (C-3'') and δ 38.5 (C-4"). The sp^2 non oxygenated quaternary carbon at δ 147.0, δ 144.7, δ 134.9 and δ 128.4 were ascribed to C-2, C-6, C-2'and C-5 respectively. The presence of two methine group on aromatic ring at position C-3 and C-4 with signal at δ 128.5 and δ 137.0 was observed on ^{13}C NMR. With the data generated the structure in Figure 8 was proposed.

This was further confirmed using the 2D-NMR including COSY, HSQC and HMBC. The COSY spectrum clearly indicates a correlation between the proton at δ 7.07 and δ 7.34. Also observed correlation in the COSY spectrum is the proton at δ 2.98 with 1.12 (H-5' with H- 6',7') and δ 2.67 with 1.08(H-6'' with H-7'',8'').

The HSQC spectrum showed the proton signal at δ 7.34, 7.07, 7.04, 3.19, 2.98, 2.70, 2.67, 2.30, 1.12 and 1.08 correlates with the carbon signals at δ 137.0, 140.1, 128.5, 24.9, 26.9, 40.7, 38.5, 19.7, 21.4 and 18.2, respectively. In the HMBC spectrum, the proton signal at δ 1.08 correlates with the carbon δ 40.7, 213.6 and 24.9. The signal at δ 2.7 correlates with the carbon signals at δ 24.9 and 213.6 (Fragment 1). The spectrum also revealed a correlation between the proton at δ 1.12 with the carbon at δ 26 and 181 (Fragment 2). While the proton at δ 2.98 correlates with the carbon at δ 181.



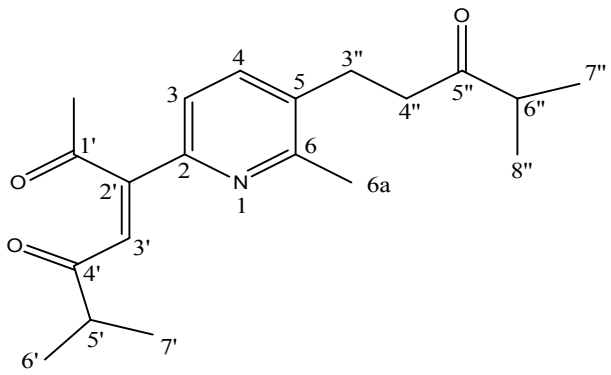
Fragment 1



Fragment 2

HMBC

Also observed correlation in the HMBC spectrum is due to the proton at δ7.04 with the carbon at δ 140.2 and 128. The proton at δ 7.34 correlates with the carbon at δ 147, δ 134 and δ 19. The data generated agreed well with the structure proposed in Figure 8.



41

Figure 8: Structure of compound **41** isolated from the root of *Ocimum cufodontii*.

Table 4: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **41**

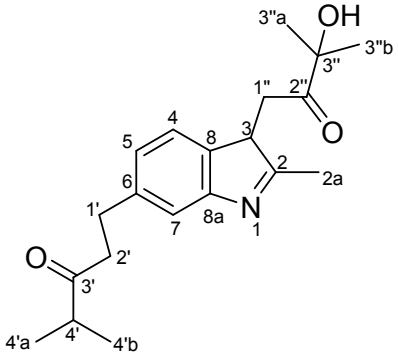
No.	$^{\delta}_{H}$ NMR data (δ in ppm, J in Hz)	$^{\delta}_{C}$ NMR data (δ in ppm)
6a	2. 3 (3H, *s*)	19.7
2	-	147.0
3	7.04 (1H, *d*, *J* = 8.42 Hz)	128.5
4	7.35 (1H, *d, J* = 8.42 Hz)	137.0
5	-	128.5
6	-	144.7
1'	-	181.2
2'	-	134.9
3'	7.04 (1H, *s*)	140.1
4'	-	182.3
5'	2.70 (1H, *septet*)	40.7
6'	1.08 (3H, d, J=6.77 Hz)	21.4
7'	1.08 (3H, d, J=6.77 Hz)	21.4
3''	3.19 (2H,t, J = 6.81 Hz)	24.9
4''	2.67 (2H,t)	38.5
5''	-	213.6
6''	2.98 (1H,*septe*t)	26.9
7''	1.13 , 6H, d, J=6.94)	18.2
8''	1.13 , 6H, d, J=6.94)	19.7

Compound 42

Compound **42** was isolated as a brown solid (29 mg) from the EtOAc root extract of *O.cufodontii*. The ES-MS spectrum revealed the molecular ion peak m/z 330.3 $[M]^+$ attributed to the molecular formula $C_{20}H_{28}O_3N$. The UV-VIS (Methanol) showed absorption maxima at 240 and 340 nm suggests the presence of chromophore in the structure.

In the ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) signals due to four methyl groups were evident at δ 1.21, δ 1.25 (6H, *s*, H-4'a,4'b) and δ 1.04, δ 0.87 (6H, *s*, H-3''a,3''b). The multiplicity observed at 4' indicated that the two methyl groups are directly attached to methine carbon. This is confirmed from the observed signals due to the methine group at δ 1.21 (1H, *septet*, H- 4'). The proton at δ 7.21 (1H, *d*, *J* = 7.6 Hz, H- 4), δ 7.08 (1H, *d*, *J* = 0.8 Hz, H-7) and δ 7.04 (1H, *d*, *J* = 7.6 Hz, H-5) were found in the aromatic region of the compound. The three methylene group at δ 1.40 (2 H, *m*, H-1'), δ 1.98 (2 H, *m ,* H-2') and δ 3.00 (2 H, *m*, H-1'') were observed from the ^{1}H-NMR. The methine proton at δ 2.90 (1 H, *m,* H-3) and the methyl proton at δ 2.40 (3 H, *s,* H-2a) was observed.

The ^{I3}C NMR spectral (Table 5) revealed the presence of two carbonyl carbon at carbon position C-3'and C-2'' with their signal observed at $^{\delta}C$ 210.5 and 203.5 respectively. The DEPT-135 revealed the presence of three methylene carbons at δ 40.3 (C-2'), δ 22.0 (C-1') and δ 29.7 (C-1''). The sp non oxygenated quaternary carbon at δ 140.5, δ 138.3, δ 137.9 and δ 121.1 were attributed for C-2, C-8a, C-6 and C-8, respectively. The presence of two methine group at position C-4' and C-3 with signal at δ 27.3, 55.7 respectively and the sp oxygenated quaternary carbon attached to the alcohol at δ 81.9 (C-3'') was observed on ^{13}C NMR. With the data generated the structure in **Figure 9** was proposed.



42

Figure 9: Structure of compound **42** isolated from the root of *Ocimum cufodontii*.

Table 5: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **42**

No.	^{1}H-NMR data of compound-42 *(δ)*	^{13}C-NMR data of compound-42 *(δ)*
1	-	-
2	-	140.5
2a	2.40 (3 H, *s,* H-2a)	17.8
3	2.90 (1 H, *m,* H-3)	55.7
4	7.21 (1H, *d*, *J* = 7.6 Hz, H-4),	141.2
5	7.04 (1H, *d* , *J* = 7.6 Hz, H-5)	130.8
6	-	137.9
7	7.08 (1H, *d*, *J* = 0.8 Hz, H-7)	127.6
8	-	138.3
8a	-	129.1
1'	1.40 (2 H, *m*, H-1')	22.0
2'	1.98 (2 H, *m*, H-2')	40.3
3'	-	210.5
4'	1.25 (1H, *septet*, H- 4')	27.3
4'a	1.21 (3H, *d*, *J* = 7.2 Hz, H-4'a)	18.9
4'b	1.25 (3H, *d*, *J* = 7.2 Hz, H-4'b)	18.2
1''	3.00 (2 H, *d*, *J* = 2.4 Hz,H-1'')	29.7
2''	-	203.5
3''	-	81.9
3''a	1.04 (3H, *s,* H-3''a)	21.4

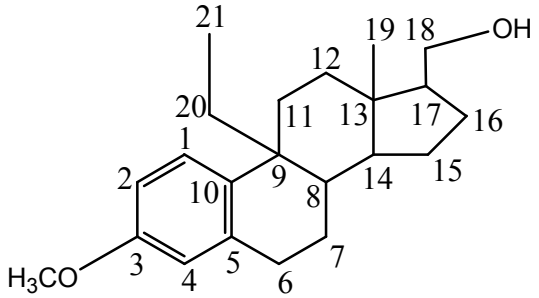
3''b	0.87 (3H, *s*, H-3''b)	21.5
OH	4.73(1H,*s*)	-

Compound 43

Compound **43** (23 mg) was isolated as a white powder from the ethyl acetate extract of the root of *Ocimum cufodontii*. The UV-Vis spectrum showed absorption band at 340 nm indicating the presence of conjugated chromphore in the structure of the compound.

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) revealed the presence of the three aromatic protons at δ 6.73 (1H), 6.37 (1H) and 6.50 (1H). The spectrum showed the presence of a methoxy group is shown at δ 3.85 (3H, *s*). The signal observed at δ 3.41 (2H, m) is due to the proton on carbon bearing oxygen. The remaining proton signals were depicted in Table 6.

The ^{13}C -NMR spectrum ($CDCl_3$, 100 MHz) show 22 carbon signals which is in agreement with the number of carbon atoms in this compound. The signals due to aromatic protons were evident at δ 135.9, 116.0, 148.4, 124.5, 145.8 and 139.9 (Table 6). The signal due to methoxy group was observed at δ 55.4.Other signal due to an oxygenated aliphatic carbon was observed at δ 61.9.



43

Figure10: The structure of compound **43** isolated from the leaves of *Ocimum Cufodontii*.

Table 6: ^{1}H and ^{13}C NMR data of compounds **43** in $CDCl_3$.

No.	^{1}H-NMR data of compound - 43 *(δ)*	^{13}C-NMR data of compound - 43 *(δ)*
1	6.73 (1H, *d*, J= 8.4 , H-1)	135.9
2	6.56 (1H, *s* , H-2)	116.0
3	-	148.4
4	6.37 (1H,*s*,H-4)	124.5
5	-	145.8
6	2.71(2H,*m*,H-6)	26.8
7	1.67 (2H,*m*,H-7)	29.6
8	1.43 (1H,*m*,H-8)	38.9
9	-	29.7
10	-	139.9
11	1.67 (2H,*m*,H-11)	27.2
12	1.38 (2H,*m*,H-12)	24.3
13	-	30.7
14	1.40 (1H,*m*,H-14)	41.8
15	1.43 (2H,*m*,H-15)	23.6
16	1.43 (2H,*m*,H-16)	22.2
17	1.67 (1H,*m*,H-17)	44.0
18	3.41 (2H,*m*,H-18)	61.9
19	1.27 (3H , *m,*H-19)	23.5
20	1.62 (2H , *m*, H-20)	28.0
21	0.89 (3H, *d* , *J* = 6.8 Hz , H-21)	14.1
CH_3O-	3.85 (3H,*s*)	55.4

4.5. GC-MS characterization of *O. cufodontii* essential oil

The leaves of *Ocimum cufodontii* was subjected to hydro-distillation to furnish 16.66% yellowish oil. Studies have reported yields of the essential oil of *Ocimum gratissimum* between 0.21 and 0.70% [97,98,99,100,101]. Hence the essential oil from *Ocimum cufodontii* is much more higher than the yield reported from *Ocimum gratissimum*. The presence of EO in the leaves of *Ocimum cufodontii* may justify the uses of this species in various forms like as spices in food industry, in aromatherapy or as a cosmetic additive and in cure of many diseases. The farmers can also make good money by selling essential oils to various kinds of industries. Thus, *O. cufodontii* is emerging as an economical crop for the farmers and traditional practitioners.

GC-MS analysis of the essential oil of the leaves of *O.cufodontii* (Figure 11) has shown the presence of 11 components and 9 of these compounds (Table 7) were identified by means of their tR, mass spectral fragmentation patterns and by comparing their mass spectra with the NIST 2005 library of mass spectra (Table7, Figure11).

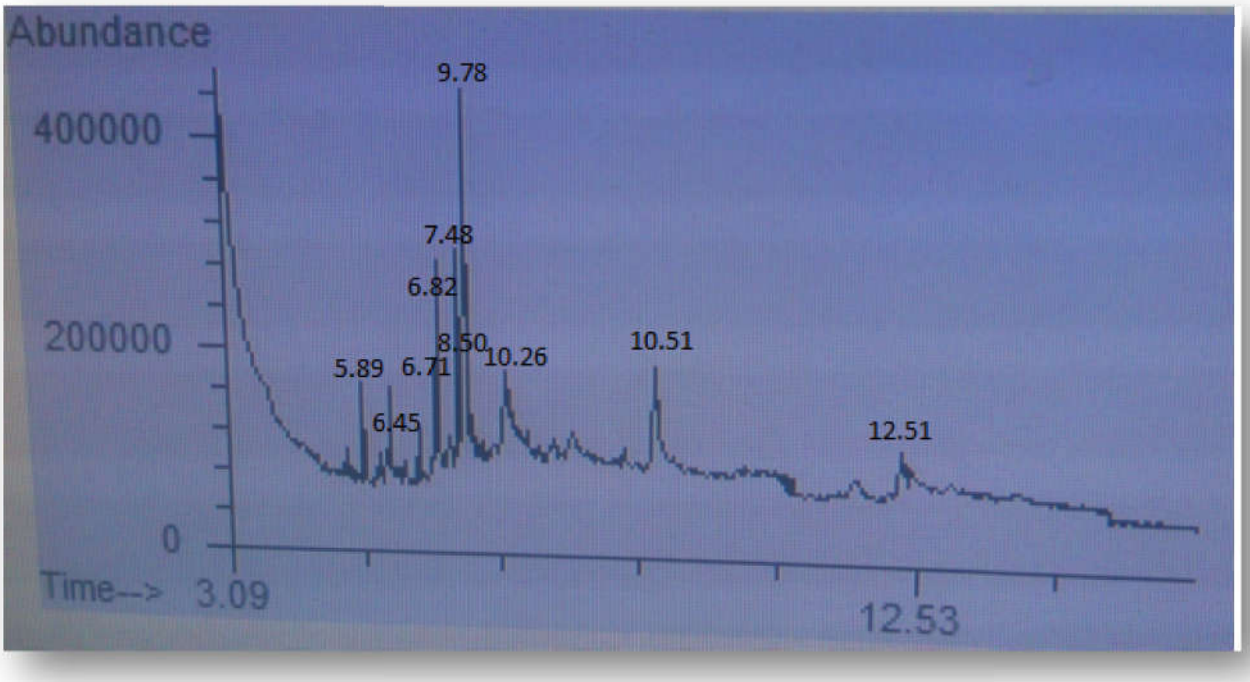


Figure 11: Typical gas chromatogram of *Ocimum cufodontii* essential oil.

Table 7: GC-MS analysis of the leaf extract of *O. cufodontii essential* oil.

tR	Compound name	Relative percentage
5.89	1-Decen-4-yne,2-nitro-	1.515
6.45	3-Cyclohexene-1-propanal	1.167
6.71	Z,Z,Z-1,4,6,9-Nonadecatetraene	30.635
6.82	Isopinocarveol	1.897
7.47	8,11-Octadecadiynoic acid,methyl ester	9.215
8.50	6,9,12-Octadecatrienoic acid,phenylmethyl ester,(Z,Z,Z)-	5.571
9.78	Caryophyllene	31.271
10.26	Methyl-10,12-pentacosadiynoate	10.978
10.51	7-Hydroxy-bicyclo[3.3.1]non-2-en-9-one	2.848
Total		**95.097**

tR: Retention time, GC-MS: Gas chromatography-mass spectroscopy, *O. cufodontii*: *Ocimum cufodontii*

Essential oils (EO) from leaves of *Ocimum gratissimum* and *Ocimum micranthum* obtained by steam distillation showed different composition by GC/MS analysis. The main components from *O. gratissimum* and *O. micranthum* showed mainly eugenol (**1**) (54.0%) and (64.8 %) respectively [102]. But the main component (Table7) from essential oils of leaves of *Ocimum cufodontii* was found to be *β*-caryophyllene (31.3%), which is a natural volatile bi-cyclic sesquiterpene that occurs in essential oils from several plants such as cinnamon, clove oil, black pepper, oregano and copaiba balsam, which have been utilized for different medicinal purposes [103, 104]. Furthermore, BCP was identified as a major constituent (35%) in the essential oil of *Cannabis sativa* L, which makes BCP an important dietary cannabinoid with high selectivity

binding to cannabinoid receptor 2 (CB2) [104]. The approximate quantity of caryophyllene in the essential oil of different *ocimum* species is given by [102] to be 5.3–10.5% from *O. gratissimum* and 4.0–19.8% from *O. micranthum*. Therefore the amount of *β*-caryophyllene present in *O. cufodontii* is superior to those reported from *Ocimum gratissimum* and *Ocimum micranthum.* Various literatures showed that *β*-caryophyllene seems to possess anti-inflamatory [105], antioxidant capacity [103], antileishmanial activity [106], induce apoptosis in tumor cells [107] and cytotoxicity effect against cancer cells [108]. Therefore the presence of *β*-caryophyllene in this plant in significant amount leads to the use of this plant species as a promising medicinal plant.

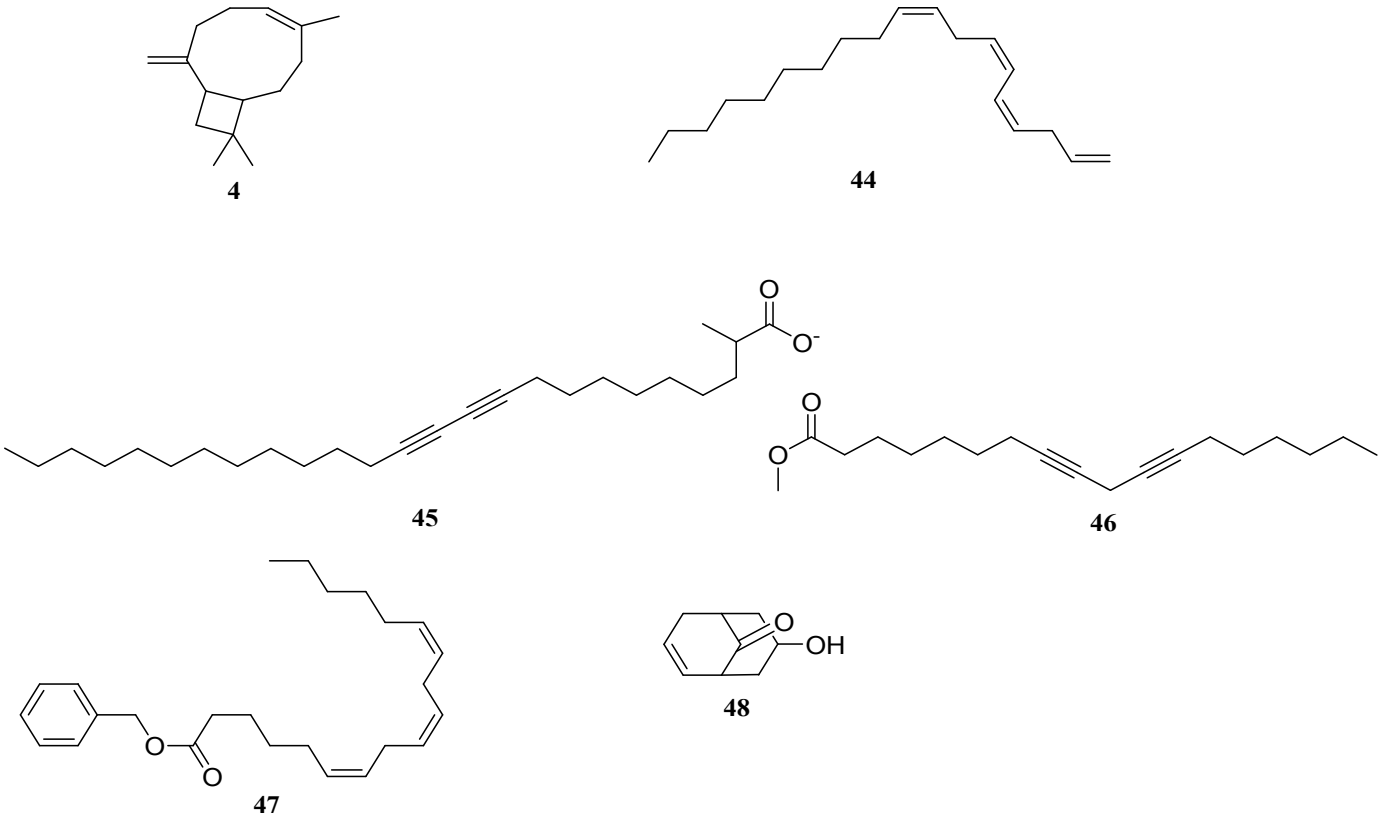


Figure12: Some major components of the essential oil of the leaves of *Ocimum cufodontii*

4.6. Antibacterial activity

In the disc diffusion assays, four bacteria species (*Escherichia coli, Klebsella pneumonia, Salmonella typhymurium* and *Staphylococcus aureus*) were chosen to determine the antimicrobial activities of hexane, EtAOc and MeOH crude extract. *E.coli*, *K.phnemonea* and *S. typhymurium* are a gram negative bacterium while the *S. aureus* species is gram positive bacteria. The crude extract of *O.cufodontii* leaves and roots were more effective against gram-positive bacteria than gram-negative bacteria. As seen from Table 8, the hexane extract of the roots and the EO from the leaves displayed bacterial inhibition zone of 17 mm and 19 mm against *S. aureus*, respectively. This clearly demonstrates that the antibacterial activity of the plant is mainly the non-polar fraction. For compounds **39** (8 mm), **41** (13 mm) and **42** (9 mm) three bacteria species (*E.coli , P.auregenesa and S. aureus*) were chosen and compounds from each extract of leaves and roots of the plant were more effective against gram-positive than gram-negative bacteria, depending on the different structural and inherited features of these two groups of bacteria .

[109] , reported the activity of *Ocimum gratissimum* extract against the *S. aureus* show 18 mm zone of inhibition, while our results from *Ocimum cufodontii* demonstrates that 17 mm and 19 mm zone of inhibition for crude and essential oil extract respectively. These established a good support to the use of this plant in herbal medicine.

Table 8: Inhibition zone of hexane, EtOAc, MeOH extract and hydro-distilled essential oil expressed in mm

Sr.No.	Code	Dose (µg/ ml)	*S. aureus*	*E.coli*	*K.phnemonea*	*S.typhyuri um*
1	HLE	5	6 mm	6 mm	6 mm	6 mm
2	EALE	5	8 mm	6 mm	8 mm	6 mm
3	MLE	5	8 mm	6 mm	6 mm	6 mm
4	HRE	5	17 mm	6 mm	6 mm	6 mm
5	EARE	5	13 mm	6 mm	8 mm	6 mm
6	MRE	5	15 mm	6 mm	6 mm	6 mm
7	Essential Oil	5	19 mm	6 mm	8 mm	8 mm
8	Compound **39**	50	8 mm	-	-	-
9	Compound **41**	50	13 mm	-	-	-
10	Compound **42**	50	9 mm	-	-	-
8	DMSO(Negative control)	5	0	0	0	0
9	Chloroamphinicol	20	28	33		

The solvent DMSO did not inhibit the growth of bacteria at the concentration used.

4.7. Antioxidant activity

In the present study different extracts of leaves and roots free radical scavenging activities of *Ocimum cufodontii* were evaluated. DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts, thus it was considered important to screen the *Ocimum cufodontii* different extracts for antioxidant activity against DPPH radical [110-111].

According to [112], *O. gratissium* extract was the most potent scavenger (81.1%) while *O.americanum, O .minimum, O. citriodorum, O. kilimandscharicum, O. grandiflorum, O. lamiifolium*and *O. selloihad* significantly lower scavenger activity as 77.4 %, 70.1 %, 60.6 %

56.2 %, 51.3 %, 46.2 % and 42.4 % respectively. In this study, *Ocimum cufodontii* which shows high inhibition of DPPH activity shows that 81.2 % in ethyl acetate leaf extract where as 90.4 % in hexane root (Table10). None of the *Ocimum cufodontii* extracts were as effective DPPH scavengers as the positive control Ascorbic acid (96.8 %). The results of DPPH-free radical scavenging assay suggest that the extracts are capable of scavenging free radicals via electron (or) hydrogen donating mechanisms and thus should be potent enough to prevent the initiation of toxic free radical mediated chain reactions in susceptible matrices eg:- biological membranes [112]. The IC_{50} values of the four different plant extracts (HRE, HLE, EARE and EALE) have been furnished in the Table 10. Highest scavenging was observed for hexane root extract (HRE) with an IC_{50} value of 1.3 µg/ml as opposed to the IC_{50} value of ascorbic acid 5.14 µg/ml, which is a well known antioxidant (Figure 13). Scavenging of DPPH radical was found to rise with increasing concentration of the extracts.

Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes [113]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [114]. Oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders such as inflammation, viral infections, autoimmune pathologies, and digestive system disorders including gastrointestinal inflammation and ulcer [115]. For instance in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role I n the long term complication of diabetes [116,117]. Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma[118]. Hence, therapy using free-radical scavenging antioxidants has potential to prevent, delay or ameliorate many of these disorders [119].

The present results suggest that all the tested plant extracts have moderate to potent antioxidant activity. Since a variety of constituents are known from the extracts studied, it becomes difficult to ascribe the antioxidant properties selectively to any one group of constituents without further studies which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active antioxidative principles present in this plant.

Table 9: Antioxidant activity of the leaves and root extracts of *O. cufodontii*

Concentrations (µg/ml)	%inhibition of HLE	IC_{50}	%inhibition of HRE	IC_{50}	%inhibition of EALE	IC_{50}	%inhibition of EARE	IC_{50}	%inhibtion of AA
12.5	51.3		50.3		42.0		45.3		
25	51.7		50.9		48.5		45.9		
50	52.8		61.2		50.7		48.8		96.8%
100	57.9	**1.97**	85.0	**1.30**	53.2	**55.83**	49.2	**116.58**	
200	65.0		90.5		68.3		52.4		

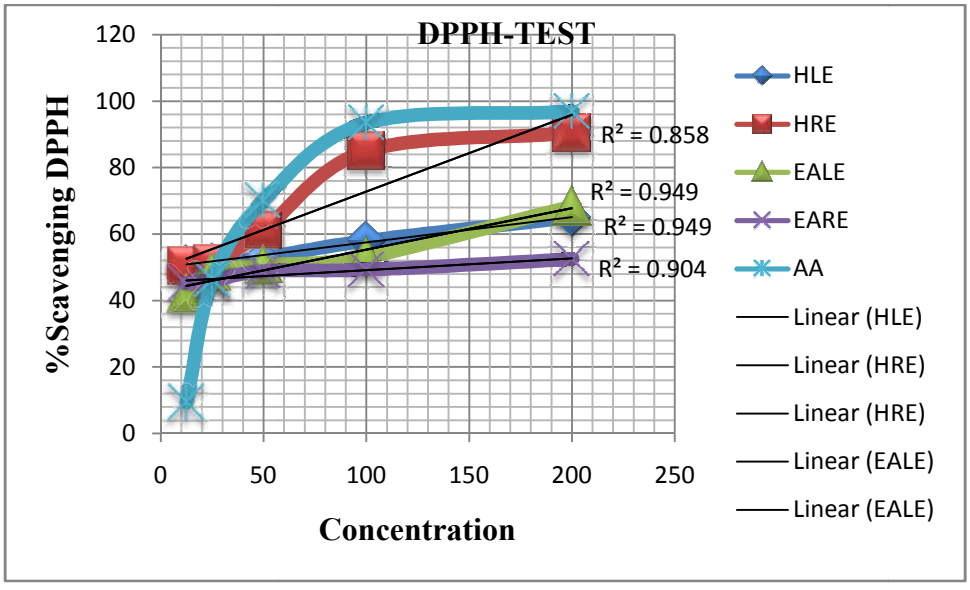


Figure 13: DPPH radical scavenging activity of crude extracts of *O.cufodontii*

5.0. SUMMARY AND CONCLUSION

Results from the study showed that phytochemicals ranging from alkaloid, tannins, steroids, terpenoids, phenols, flavonoids, anthraquinone glycosides, saponin glycosides, cardiac glycosides and reducing sugars were present in the study as represented in Table 1.

The antibacterial results of disk diffusion tests showed the inhibition zones of *S. aureus* 19.0 mm for essential oil, 17 mm for hexane extract of the root and 15 mm for MeOH root extract. This clearly demonstrates that the antibacterial activity of the plant is mainly the non-polar fraction.
For betulinic acid **(39)** (8 mm), compound **41** (13 mm) and compound **42** (9 mm) bacteria species (*E.coli , P.auregenesa and S. aureus*) were used and compounds from each extract of leaves and roots of the plant were more effective against gram-positive than gram-negative bacteria, depending on the different structural and inherited features of these two groups of bacteria . The results of this study showed the presence of bacteriostatic effects of essential oil, the hexane and methanol root extract respectively of *Ocimum cufodontii* on gram-positive bacteria, *Staphylococcus aureus*. These established a good support to the use of this plant in herbal medicine.

The antioxidant activities of all the tested samples were concentration-dependent. Based on the results obtained, we can conclude that among the four fractions, the HRE one showed the highest scavenging activity, with an IC_{50} value of 1.30 µg/ mL. Hence, HLE with an IC_{50} value 1.97 µg/mL showed more efficient radical-scavenging abilities than EALE and EARE with an IC_{50} value of 55.83 µg/ mL and 116.58 µg/mL respectively. The hexane root and leaves extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and the healthy food industry.

References

1. Cotton, C. M. (**1996**). Ethnobotany: Principles and applications. Chichester, New York: John Wiley and Sons Ltd.
2. WHO, Traditional medicines: global situation, issues and challenges, Geneva, 2011.
3. Ermias L, Ensermu K, Tamrat B and Haile K (**2008**). An ethnobotanical study of medicinal plants in Mana District. Southeastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*; **14**: 4-10.
4. Dery, B. B.; Ofsynia, R. and Ngatigwa, C. (**1999**). Indigenous knowledge of medicinal trees and setting priorities for their domestication in Shinyanga region: International Center for Research in Agroforestry; Nairobi.
5. Lino A, Deogracious O (**2006**). The *in vitro* antibacterial activity of Annona senegalensis, Securidacca longipendiculata and Steanotaenia araliacea Ugandan medicinal plants. *Afr Health Sci* ;**6**: 31-5.
6. Nacoulma-Ouedraogo, O. G. *Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso, cas du plateau central, Tome I et II* Thèse de Doctorat d'Etat. thesis, Université de Ouagadougou., (**1996**).
7. Herbon JB(**1998)**. Phytochemical Methods: A guide to Modern Techniques of Plant Analysis, 2nd Edition Hall, New York; 5-11.
8. Farnsworth N. R (**1994**). Ethnobotany and the search for new drugs, Ciba Foundation Symposium 185.Ethnopharmacology and drug development, Chichester, Uk Prance GT, John Wiley and Sons; pp. 42-59.
9. Lanfranco, G (**1999**). Invited review article on traditional medicine. *Electronic Journal of Biotechnology;* **2**: 1-3.
10. Demma J , Engidawork E. and Hellman B (**2009**). Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *Journal of Ethnopharmacology,* **122**, 136-142.
11. Tatyana V., Ramón Morales, Krestovskaja J. Alan Paton, OlofRyding P (**2004**)."Labiatae"pages. In: The Families and Genera of Vascular Plants, volume VII; 167-275.
12. Cseke LJ, Kirakosyan A, Kaufman PB, Sara L, James AW, Duke, *et al*. (**2006)**. Natural Products from Plants. Second edition.

13. Steenkamp, V.(**2003**). Traditional herbal remedies used by South African women in gynaecological complaints. *Journal of Ethnopharmacology,* **86**, 97-108.
14. WHO. Fact Sheet, Traditional Medicine, Geneva, May 2003.
15. Chirchir, J.; Mungai, G. and Kariuki, P. (**2006**).Indigenous knowledge and conservation of natural resources: Resource medicinal plants utilisation in Eastern Africa. Proceedings of national museums of Kenya first scientific conference, 15th-17th Nov 2006;pp. 106-111.
16. Njoroge, N. G.; Bussmann, W.; Rainer, G. B.; Newton, L. E. and Ngumi, V. W. (**2004**). Utilisation of weed species as sources of traditional medicines in central Kenya.*Lyonia,***72**, 71-87.
17. Runyoro, D. K. B. ; Ngassapa, O. D.; Matee, M. I. N.; Joseph, C. C. and Moshi, M. J. (**2006**). Medicinal plants used by the Tanzanian traditional healers in the management of Candida infections. *Journal of Ethnopharmacology,* **111**, 284-294.
18. Cunningham, A. B. (**1996**). People, park and plant use. Recommendations for multipleuse zones and development alternatives around Bwindi Impenetrable National Park, Uganda. People and plants working paper 4. United Nations Educational, Scientific and Cultural Organization, Paris.
19. Tapsoba, H. and Deschamps, J. P. (**2006**). Use of medicinal plants for the treatment of oral diseases in Burkina Faso.*Journal of Ethnopharmacology* ,**104**, 68-78.
20. Noumi, E. and Yomi, A. (**2001**). Medicinal plants used for intestinal diseases in Mbalmayo Region, Central Province, Cameroon.*Fitoterapia,* **72**, 246-254.
21. Weniger, B. ; Lagnika, L.; Vonthron-Sénécheau, C.; Adjobimey, T.; Gbenou, J.; Moudachirou, M.; Brun, R.; Anton, R. and Sanni, A. (**2004**). Evaluation of ethnobotanically selected Benin medicinal plants for their in vitro antiplasmodial activity. *Journal of Ethnopharmacology,***90**, 279-284.
22. Abebe, D. (**1996**). The role of herbal remedies and the approaches towards their development. In:Proceedings of the workshop on development and utilization of herbal remedies in Ethiopia, Nazareth; pp. 29-35.
23. Abebe, D. (**2001**). The role of medicinal plants in healthcare coverage of Ethiopia, the possible benefits of integration. In: Zewdu, M. ed. Conservation and sustainable use of

medicinal plants in Ethiopia, Proceedings of the National workshop, 28 April - 01 May 1998, Addis Ababa, Institute of Biodiversity Conservation and Research; pp. 6-21.

24. Tadeg, H.; Mohammed, E.; Asres, K. and Gebre-Mariam, T. (**2005**).Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacolgy,* **100**, 168-175.
25. Gill, NS. ; Dhawan, S. ; Jain, A; Arora, R. and Bali, M. (**2012**). "Antioxidant and anti-ulcerogenic activity of wild *Punica granatum* ethanolic seed extract", *Res. J. Med. Plant*,**6**,47-55.
26. Gill ,NS. And Bali, M. (**2012)**. "Evaluation of antioxidant, antiulcer activity of 9-beta-methyl-19-norlanosta-5-ene type glycosides from *Cucumis sativus* seeds". *Res. J. Med.Plant*,**6**, 309-317.
27. Gill, NS. ; Sood , S. ; Bansal , S. ; Muthuraman, A. and Bali ,M. (**2009**). "Therapeutic potential of*Citrus medica* L. Peel extract in carrageenen induced inflammatory pain in rat``, *Res. J. Med. Plant*, 3**(4)**, 123-133.
28. Gill,NS. ; Kaur, S.; Arora, R. and Bali, M. (**2011**). "Screening of antioxidant and antiulcer potential of *Citrullus colocynthis* methanolic seed extract". *Res. J. Phytochemi*., 5 **(2)**, 98-106.
29. Arora, R.; Kaur, M. and Gill, NS. (**2011**). "Antioxidant activity and pharmacological evaluation of *Cucumis melo* var.Agrestis methanolic seed extract". *Res. J Phytochemi.*, 5**(3**), 146-155.
30. Kokate, C K.; Purohit, A P. and Gokhale, S B. (**2002**). Pharmacognosy, 20thEdn , Nirali prakashan, Pune,108-109.
31. Leung, A.Y. and Foster, S. (**1996**) *Encyclopedia of Common Natural Ingredients used in foods, drugs, and cosmetics*,2nd ed., John Wiley & Sons, New York, USA.
32. Paton, A. (**1992**). A synopsis of *Ocimum* L. (Labiatae) in Africa.*Kew Bull*; **47**: 405–437.
33. Krishnaraju A. V, Rao T. V. N and Sundararaju D (**2005**). Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering;* **2**, 125-134.
34. Roja G and Rao P. S (**2000**).Anticancer compound from tissue cultures of medicinal plant. *Journal of Medicinal Plants, Spices and Medicinal Plants;* **7**, 71-102.

35. Demissew S and Asfaw N (**1994**). Some useful indigenous labiates from Ethiopia.*Lamiales Newsletter;* **3**: 5–6.
36. Sarac, N. and A. Ugur (**2007**). Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. *EurAsia J. Bio. Sci*., **1**:28-34.
37. Giuliani, C. And L. Maleci Bini (**2008**). Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. *Plant systematic and Evolution*, **276**:199-208.
38. Ozkan, M (**2008**). Glandular and eglandular hairs of salvia recognita Fisch.And Mey.(Lamiaceae) in Turkey. *Bangladesh Journalof Botany*, **37**:93-95.
39. Gaur S and Kaushik P (**2011)**. "Influence of edaphic factors on distribution of mycorrhiza associated with medicinal plants in India central Himalayas". *J. Bio. Sci*; **11**: 349-358.
40. Paton, A. W.; Woodrow, M. C.; Doyle, R. M.; Lanser, J. A. & Paton, J. C. (**1999**).Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking eae responsible for a cluster of cases of hemolytic-uremic syndrome. *J Clin Microbiol*.**37**:3357–3361.
41. Chang J. (**2000**). Medicinal herbs: Drugs or dietary supplements. *Biochem Pharmacol*, **59**, 211–219.
42. Darrah, H.H. (**1998**). The cultivated Basil.Buckeye Printing.Independence, MO. 68 p.
43. Kirtikar KR, Basu BD(**1965)**. *Ocimum sanctum* in Indian medicinal plants (Published by LB Basu. Allahabad).
44. Pandey BP(**1990**). Anita in economic botany (Published by Chand & Company Ltd. Ramnagar. New Delhi , 294.
45. Gupta SK, J Prakash, S Shrivastava (**2002**). Validation of claim of tulsi *Ocimum sanctum* as medicinal plant. *Indian J. Experimental biology*; **40(7):**765-773.
46. Narwal S, Rana AC, Tiwari V, Gangwani S and Sharma R (**2011)**. "Review on chemical constituents and pharmacological action of *Ocimum kilimandscharicum*". *Indo.Global J. Pharmaceu. Sci*; **1**(**4**): 287-293.
47. Joshi B, Sah GP, Basnet BB, Bhatt MR and Sharma D (**2011**). "Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum*

sanctum (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem)", *J. Microbiol.Antimicrobials*; **3**: 1-7.

48. SmithRL,CohenSM, Doull J, Feron VJ and Goodman JI (**2005**). "A procedure for the safety evaluation of natural flavor complexes used as ingredients in food", Essential oils.*J. Food Chem. Toxicol;* **43**: 345-363.
49. A.J. Paton, G. Bramley, O. Ryding, R.M. Polhill, Y.B. Harvey, M. Iwarsson, F. Willis, P.B. Phillipson, K. Balkwill, C.W. Lukhoba, D.F. Otieno, & R.M. Harley (**2009**). Flora of Tropical East Africa.
50. El-Said, F., Sofowora, E.A., Malcolm, S.A. and Hofer, A. (**1969**). An investigation into the efficacy of *Ocimum gratissimum* as used in Nigerian native medicine.*Planta Medica*, **17**: 195–200.
51. Thomas, O.O (**1989**) .Re-examination of the antimicrobial activities of *Xylopia aethiopica, Carica papaya, Ocimumgratisssimum Jatropa curcas*.*Fitoterapia*, **LX**: 147–155.
52. Silva L.L, Heldwein C.G, Reetz L.G.B, Hörner R, Mallmann C.A and Heinzmann B.M. (**2010**). Chemical composition, antibacterial activity *in vitro* and brine-shrimp toxicity of the essential oil from inflorescences of *Ocimum gratissimum* L., [In Process].*Brazilian Journal of Pharmacognosy;* **20**(**5**):700-05.
53. Carovic-Stanko K, Orlic S, Politeo O, Strikic F, Kolak I, Milos M and Satovic Z (**2006**). "Composition and antibacterial activities of essential oils of seven *Ocimum* taxa." *Food Chem*; **119**(**1**): 196-201.
54. Navarro M. C, Montilla M. P, Cabo M.M, Galisteo M, Cáceres A. C, Morales I. Berger. (**2003**). Antibacterial, antiprotozoal and antioxidant activity of five plants used in izabal for infectious disease. *phytotherapy research*;**17**:325-29.
55. Bassole IHN, Nebie R, Savadogo A, Ouattara CT, Barro N and Traore SA (**2005**). "Composition and antimicrobial activities of the leaf and flower essential oils of Lippia chevalieri and *Ocimum canum* from Burkina Faso" . *Afr. J. Biotec*h; **4**: 1156-1160.
56. Prashar R, Kumar A, Banerjee S and Rao A.R (**1994**). Chemopreventive action by an extract from *Ocimum sanctum* on mouse skin papilloma genesis and its enhancement of

skin glutathione S-transferase activity and acid soluble sulfhydryl level. *Anti-cancer Drugs*; **5**: 567–572.

57. Gajul D, Verghese M, Boateng J,Shackelford L, MentreddySR, Sims C, Asiamah D and Walker LT (**2010**). ``Basil (*O. basilicum* and*O. tenuiflorum*) reduces azoxymethane induced colon tumors in fisher 344 male rats``. *Res. J. Phytochem*; **4**:136-145.
58. Ekunwe S.I, Thomas M.S, Luo X, Wang H, Chen Y, Zhang X, Begonia G.B(**2010**). Potential cancer-fighting *O. gratissimum* leaf extracts: increased anti-proliferation activity of partially purified fractions and their spectral fingerprints. *Ethnicity & disease;* **20**: 1 Suppl 1 (S1-12-6).
59. Chattopahdyay R.R (**1993**). Hypoglycemie effect of *Ocimum sanctum* leaf extract in normal and streptozotocin diabetic rats.*Indian J. Exp. Biol*; **31**: 891–893.
60. Rai V, lyer U and Mani U.V (**1997**).Effect of Tulsi (*O. sanctum*) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. *Plant Foods for Human Nutrition*; **50**: 9–16.
61. Sarkar A, Lavania S.C, Pandey D.N and Pant M.C (**1994**). Changes in the blood lipid profile after administration of *Ocimum sanctum*(Tulsi) leaves in the normal albino rabbits. *Indian J. Physiol. Pharmacol*; **38**:311–312.
62. Grover JK, Yadav S and Vats V (**2002**) Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*; 81(**1**): 81-100.
63. Raja, RR. (**2012)** "Medicinally Potential Plants of Labiatae (Lamiaceae) Family: An Overview", *Res. J. of Med. Plant*, 6**(3**), 203-213.
64. Nyarko AK, Asare-Anane H, Ofosuhene M, Addy ME, Teye K and Addo P **(2002**). "Aqueous extract of *Ocimum canum* decreases levels of fasting blood glucose and free radicals and increases antiatherogenic lipid levels in mice". *Vascular Pharmacol*; **39(6):**273-279.
65. Sakina M.R, Dandiya P.C, Hamdard M.E and Hameed A (**1990**).Preliminary psychopharmacological evaluation of *Ocimum sanctum* leaf extract.*J. Ethnopharmacol*; **28**: 143–150.
66. Yanpallewar SU, Sunita R, Kumar M and Acharya SB (**2004**). "Evaluation of antioxidant and neuroprotective effect of *Ocimum sanctum* on transient cerebral

ischemia and long-term cerebral hypoperfusion. *Pharmaco.Biochem.Behav* ; **79**(**1**): 155-164.

67. Mbata TI and Saikia A (**2009**).“Antibacterial activity of essential oil from *ocimum gratissimum* on Listeria monocytogenes”.*Inter. J. Food Safety*; **7**: 15-19.
68. Akhtar M.S and Munir M (**1989**).Evaluation of the gastric antiulcerogenic effects of *Solanum nigrum*, *Brassica oleraceae* and *Ocimum basilicumin* rats.*J. Ethnopharmacol*; **27**: 163–176.
69. Sakr SA, Abd SF, Osman M, Kandil AM and Helmy MS (**2011**). “Ameliorative effect of aqueous leave extract of *Ocimum Basilicum* on Ccl_4 -Induced Hepatotoxicity and Apoptosis in Albino Rats*”, J. Amer. Sci*; **7**:116-127.
70. Akhtar M.S.A, Akhtar A.H and Khan M.A (**1992**). Antiulcerogenic effects of *Ocimum basilicum* extracts, volatile oils and flavonoid glycosides in albino rats. *Int. J. Pharmacognosy*; **30**: 97–104.
71. Mandal S, Das D.N, De K, Ray K, Roy G, Chaudhuri S.B, Sahana C.C and Chowdhuri M.K (**1993**) . *Ocimum sanctum* Linn.A study on gastric ulcération and gastric secretion in rats.*Indian J. Physiol. Pharmacol*; **37**: 91–92.
72. Singh S and Majumdar D.K (**1997**). Evaluation of anti inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J. Exp. Biol*; **35**: 380–383.
73. Lin CC, lin JK and Chang CH (**1995**). Evaluation of hepatoprotective effects of “Chhit-Chan- Than” from Taiwan. *Int. J. Pharmacognosy*, **33**:139–143.
74. Mukazayire MJ, Allaeys V, Calderon PB, Stevigny C, Bigendako MJ and Duez P (**2009**). “Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on *in vivo* (guinea pigs barbiturate induced sleeping time) and *in vitro* (rat precision-cut liver slices, PCLS) models”, *Experimental Toxicol. Pathol*; **62**: 289–299.
75. Taie HAA, Salama ZAR and Radwan S (**2010**). “Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and Bio-organic fertilization. *Not. Bot. Hort. Agrobot. Cluj*”; **38**(**1**): 119-127.
76. Seyoum A, Killeen GF, Kabiru EW, Knols BGJ and Hassanali A (**2003**). “Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. *Trop”, Med. Int. Health*; **8**: 1005-1011.

77. Mwangi E.N, Hassanali A, Essuman S, Myandat E, Moreka L and Kimondo M (**1995**). Repellant and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks.*Exp. Appl. Acaro/ogy*; **19**: 11–18.
78. Chagonda LS , Makanda CD and Chalchat JC (**2000**). "The essential oils of *Ocimum canum* sims (basilic camphor) and *Ocimum urticifolia* roth from Zimbabwe", *Flavour Fragr. J;***15**: 23–26.
79. Kashyap CP, Ranjeet K, Vikrant A and Vipin K (**2011**). "Therapeutic potency of *Ocimum Kilimandscharicum* Guerke-a review", *Global J. Pharma.*; **5**(**3**):191-200.
80. Tarchoune I , Incerti A, Lachaal M, Ouerghi Z, Izzo R & Navari-Izzo F (**2009**). Relations between antioxidant activity and salinity in basil (*Ocimum basillicum*Mill), *Agrochimica*; **LIII**, 56-64.
81. Balanehru S and Nagarajan B (**1994**). Triterpenes intervene in adriamycin-induced histamine release in rodents. *Med. Sci. Res.*; **22**: 357–359.
82. Javanmardi J, A.Khalighi A, Kashi Bais H. P and Vivanco J.M (**2002**). Chemical characterization of basil (*Ocimum basilicum L.*) found in local accessions and used in traditional medicines in Iran. *J. Agric. Food Chem.*; **50**(**21**):5878-5883.
83. Zhang JW, Li SK, Wu, WJ. (**2009**). "The main chemical composition and *in vitro* antifungal activity of the essential oils of *Ocimum basilicum* Linn. Var.pilosum (Willd.) Benth", *Molecules*; **14**:273-278.
84. http://en.wikipedia.org/wiki/Ocimum.
85. Siddiqui AA, Ali M (**1997**). Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, pp. 126-131.
86. Siddiqui S, Verma A,Rather AA, Japeen F, Meghvansi MK(**2009**). Preliminary phytochemicals analysis of some important medicinal and aromatic plants.*Advances in biological research*; **3**(**5-6**): 188-195.
87. Iyengar MA (**1995**). Study of Crude Drugs.8th ed., Manipal Power Press, Manipal, India. pp. 2.
88. Anon (**1996**). The Indian pharmacopoeia. Government of India, New Delhi. Ministry of Health and family welfare.
89. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (**2001**).Antioxidant principles from Bauhinia terapotensis. *J. Nat. Prod;* **64**: 892-895

90. Igoli JO, Gray IA (**2008**). Friedelanone and other triterpenoids from *Hymenocardia acida*.*International Journal of Physical Sciences*; **3(**6**)**:156-158.
91. Nazma P, Mohammad SR, Mohammad SI, Mohammad AR (**2009**). Chemical and biological investigations of *Dillenia indica* Linn. *Journal of the Bangladesh Pharmacological Society*; **4**: 122-125.
92. Ghias U, Bina SS, Muhammad A, Anwar S, Ashfaq A, Ala U (**2011**). Chemical constituents and phytotoxicity of solvent extracted fractions of stem bark of *Grewia optiva* drummond ex burret. *Middle-East Journal of Scientific Research*; **8(**1**)**:85-91.
93. Siddique S, Hafeez F, Begum S, Siddique BS (**1988**). Oleanderol, a new pentacyclic triterpene from the leaves of Nerium oleander. *J Nat Prod*; **51**(2): 229-233.
94. Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR, Cordell GA, Beecher CW, Fong HH, Kinghorn AD, Brown DM, Wani MC, Wall ME, Hieken TJ, Das Gupta TK, Pezzuto JM (**1995**). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Nat Med*.; **1**: 1046-51.
95. Khare CP (**2008**). Indian Medicinal Plants: An Illustrated Dictionary. Springer-Verlag Heidelberg, New York, USA: 443-447.
96. A. I. Scott (**1964**). Interpretation of Ultraviolet Spectra of Natural Products. Oxford: Pergamon.
97. Dubey NK, Tiwari T, Mandin D, Andriamboavonjy H, Chaumont J-P(**2000**). Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). *J Fitot*.; **71**(5): 567-9.
98. Luz JMQ, Ehlert PAD, Innecco R(**2009**). Horário de colheita e tempo de secagem da alfavaca-cravo. *Hortic bras*.; **27**(1): 539-42.
99. Cortez DAG, Cortez LER, Pessini GL, Doro DL, Nakamura CV(**1998**). Análise do óleo Essencial da alfavaca *Ocimum gratissimum* L. Arquivos de Ciências da Saúde da UNIPAR.; **2**(2): 125-7.
100. Nakamura CV, Ueda-Nakamura T, Bando E, Melo AFN, Cortez DAG, Dias Filho BP(**1999**). Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Mem Inst Oswaldo Cruz*.; **94**(5): 675-8.
101. Silva LL, Heldwein CG, Reetz LG, Hörner R, Mallmann CA, Heinzmann BM(**2010**). Composição química, atividade antibacteriana in vitro e toxicidade em

Artemia salina do óleo essencial das inflorescências de *Ocimum gratissimum* L, Lamiaceae. *Rev bras farmacogn*.; **20**(5): 700-5.

102. Silva, Maria Goretti de Vasconcelos; Matos, Francisco José de Abreu; Lopes, Paulo Roberto Oliveira; Silva, Fábio Oliveira; Holanda, Márcio Tavares (**2004**). "Composition of essential oils from three *Ocimum* species obtained by steam and microwave distillation and supercritical CO_2 extraction" . *Arkivoc*.(**vi**): 66–71.

103. Calleja MA, Vieites JM, Montero-Meterdez T, Torres MI, Faus MJ, Gil A, *et al* (**2013**). The antioxidant effect of *β*-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *British J nutr Br J Nutr*; **109**: 394-401.

104. Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, (**2008**). *β*-caryophyllene is a dietary cannabinoid. *Proc Natl Acad Sci USA*; **105**: 9099-9104.

105. Saad S. Dahham, Yasser M. Tabana, Mohamed B. Khadeer Ahamed , Amin M.S. Abdul Majid (**2015**). *In vivo* anti-inflammatory activity of *β*-caryophyllene, evaluated by molecular imaging. *Molecules & Medicinal Chemistry,* **1**: e1001.

106. Soares DC, Portella NA, Ramos MFDS, Siani AC, Saraiva EM (**2013**). Trans-β-Caryophyllene: An Effective antileishmanial compound found in commercial copaiba oil (*Copaifera* spp.). Evidence-based complementary and alternative medicine Evid Based Complement Alternat Med; 1-13.

107. Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S (**2012**). β-Caryophyllene, a compound isolated from the biblical balm of gilead (*Commiphora gileadensis*), is a selective apoptosis inducer for tumor cell lines. Evidence-based complementary and alternative medicine Evid Based Complement *.Alternat Med*; 1-8.

108. Jun NJ, Mosaddik A, Moon JY, Jang KC, Lee DS, Ahn KS (**2011**). Cytotoxic activity of β-caryophyllene oxide isolated from Jeju Guava (Psidium cattleianum Sabine) leaf. *Rec Nat Prod*; **5**: 242-246.

109. Ajose and Okozi (**2017**). Antibacterial Activity of *Ocimum gratissimum* L. against Some Selected Human Bacterial Pathogens. *JPRI*, **20**(1): 1-8.

110. Koleva I I, Van Beek T A, Linseen J P H, de Groot A and Evestativa L N(**2002**). Screening of plant extracts for antioxidant activity.Acomparative study on three testing methods. *Phytochemical analysis*; **13**: 8-17.

111. Suresh P K, Sucheta S, Sudarshana V D, Selvamani P and Latha S (**2008**). Antioxidant activity in some selected Indian medicinal plants. *African Journal of Biotechnology*; **7**: 1826-1828.

112. Lukmanul Hakkim F, Gowri Shankar C, Girija S (**2007**). Chemical composition and antioxidant property of holy basil (*Ocimum sanctum* L.) leaves, stems and inflorescence and their *in vitro* callus cultures. *Journal of Agricultural and Food chemistry*; **55**: 9109-9117.

113. Rahman MAA, Moon SS (**2007**).Antioxidant polyphenol glycosides from the Plant Draba nemorosa. *Bull. Korean Chem.Soc*; **28(5):** 827-831.

114. Akter R, Hasan SMR, Siddiqua SA, majumder MM, Hossain MM, AlamMA, Haque S, Ghani A (**2008**). Evaluation of analgesic and antioxidant potential of the leaves of *Curcuma alismatifolia gagnep*. S. *J.Pharm. Sci*; **1(**1 &2**):** 3-9.

115. Aruoma OI (**2003**). Methodological considerations for characterizing potential antioxidant actions of bioactive components in food plants. *Mutat. Res*;**523 – 524**:9-20.

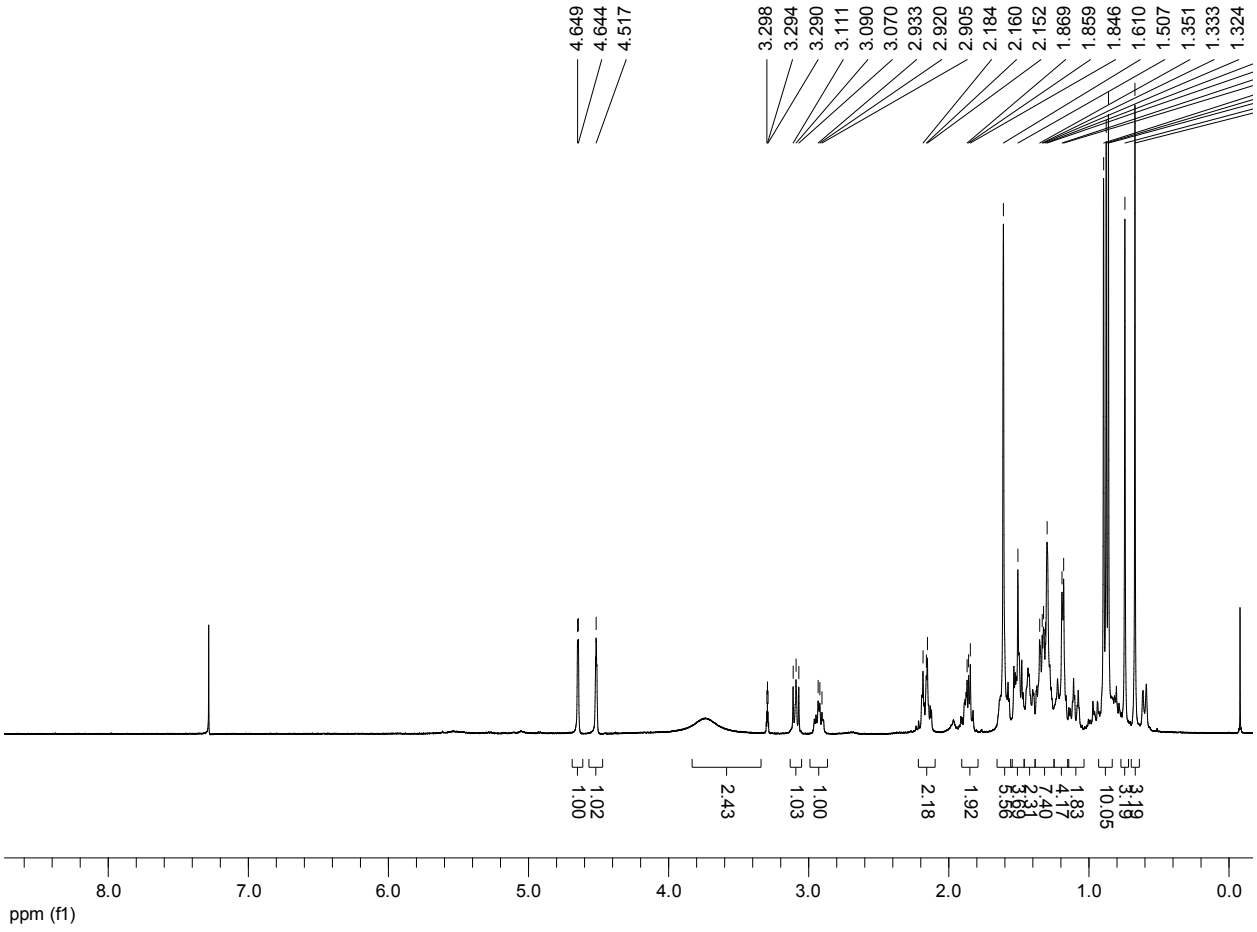
116. Sabu MC, Kuttan R (**2002**). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property.*J. Ethnopharmacol*; **81:** 155-160.

117. Atawodi SE (**2005**). Antioxidant potential of African medicinal plants.*Af. J. Biotech*; **4 (2):** 128-133.

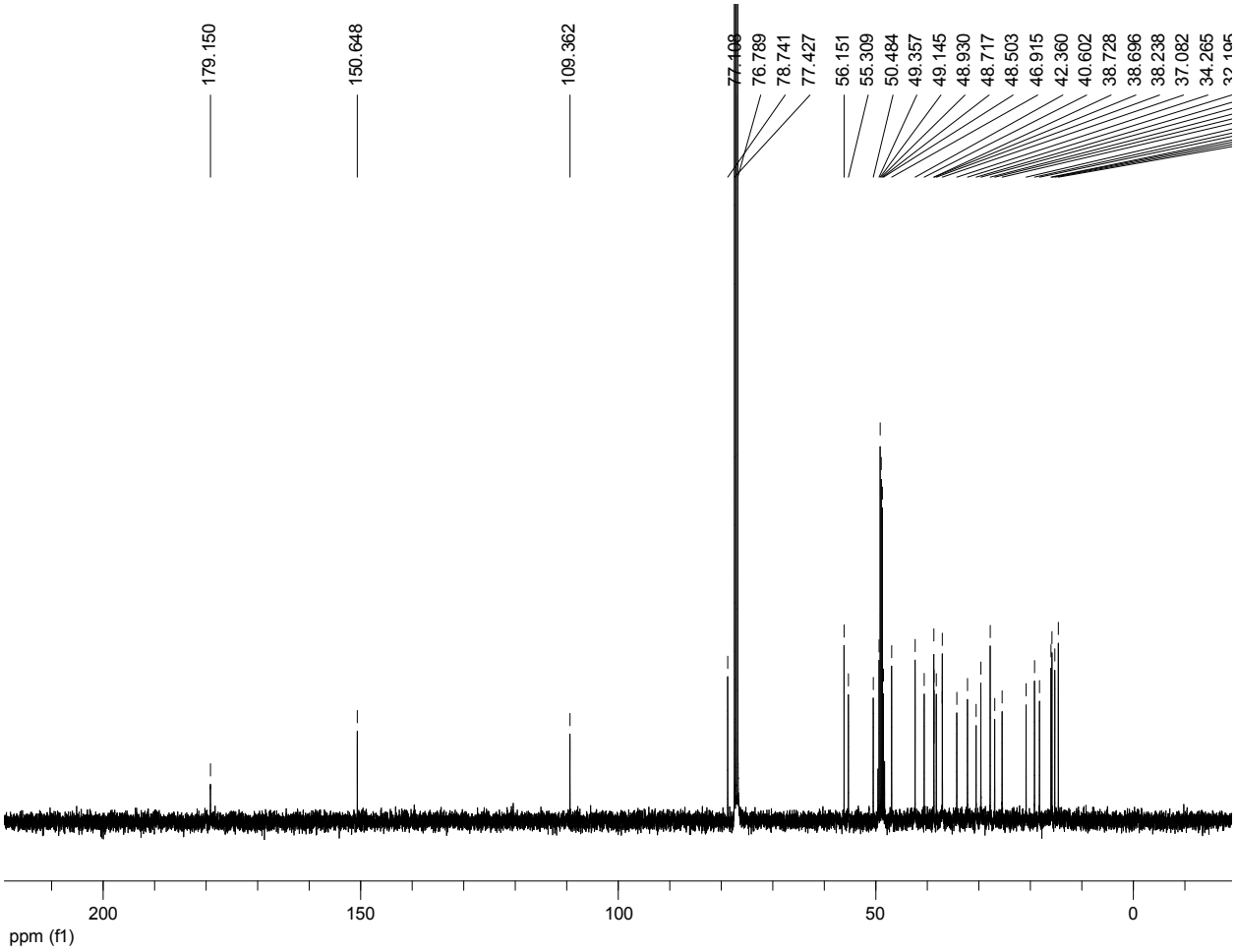
118. Tsao AS, Kim ES, Hong WK (**2004**). Chemoprevention of Cancer. *CA Cancer J. Clin*. 54: 150-180.

119. Delanty N, Dichter MA (**2000**). Antioxidant therapy in neurologic diseases.*Arch. Neurol*; **57(9):**1265-1270.

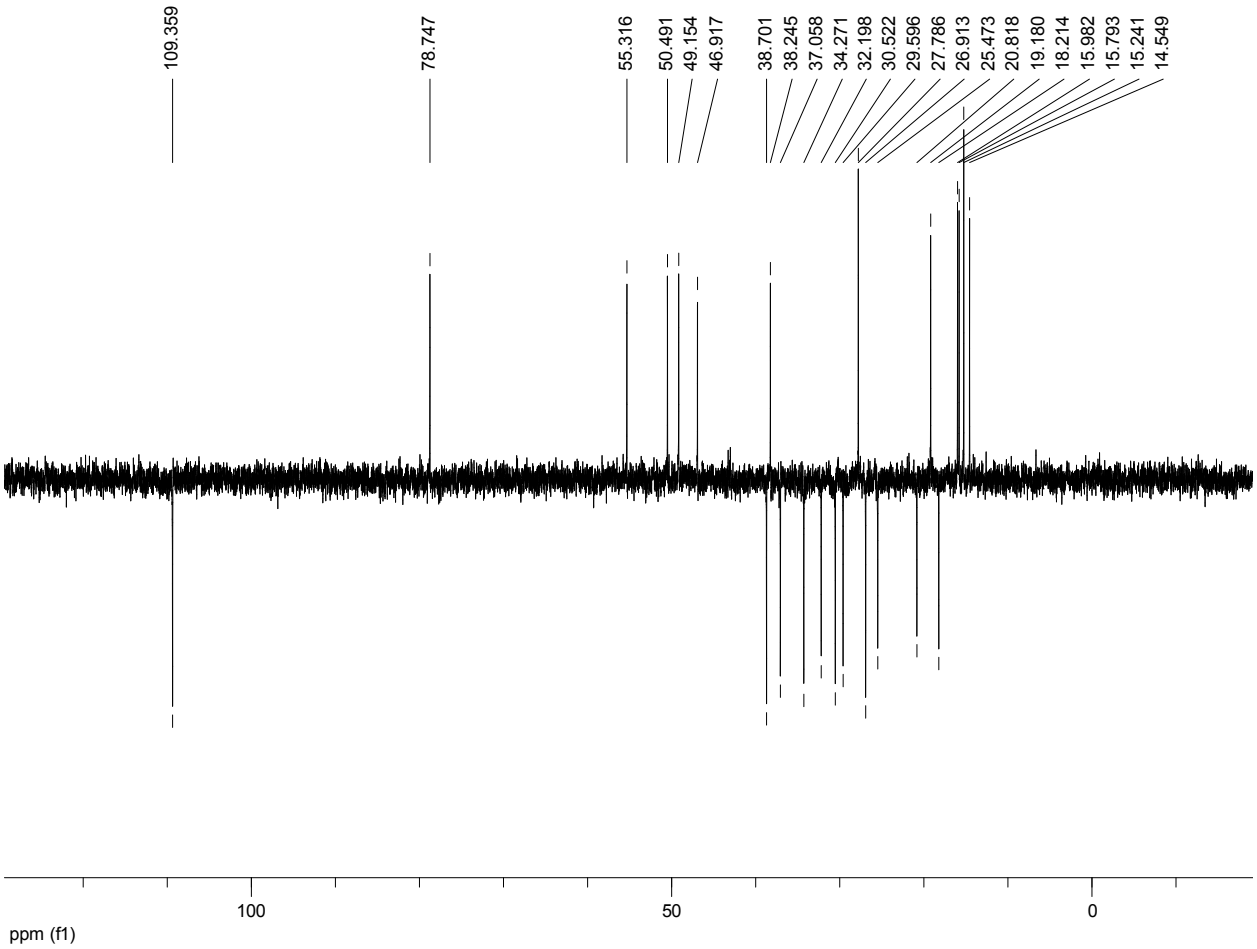
Appendix 1A: ^{1}H-NMR spectrum of betulinic acid **(39)**



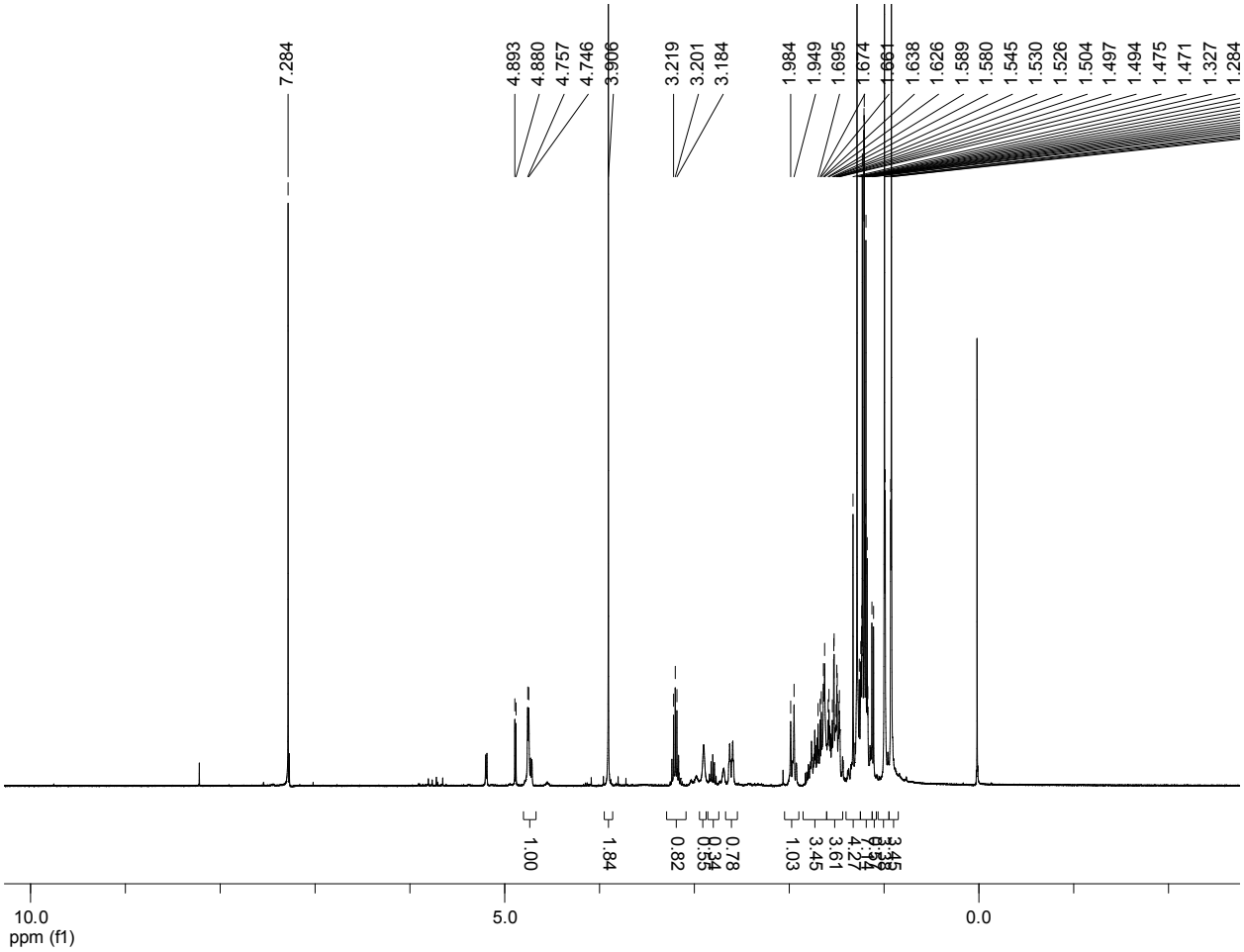
Appendix 1B: ^{13}C-NMR spectrum of betulinic acid **(39)**



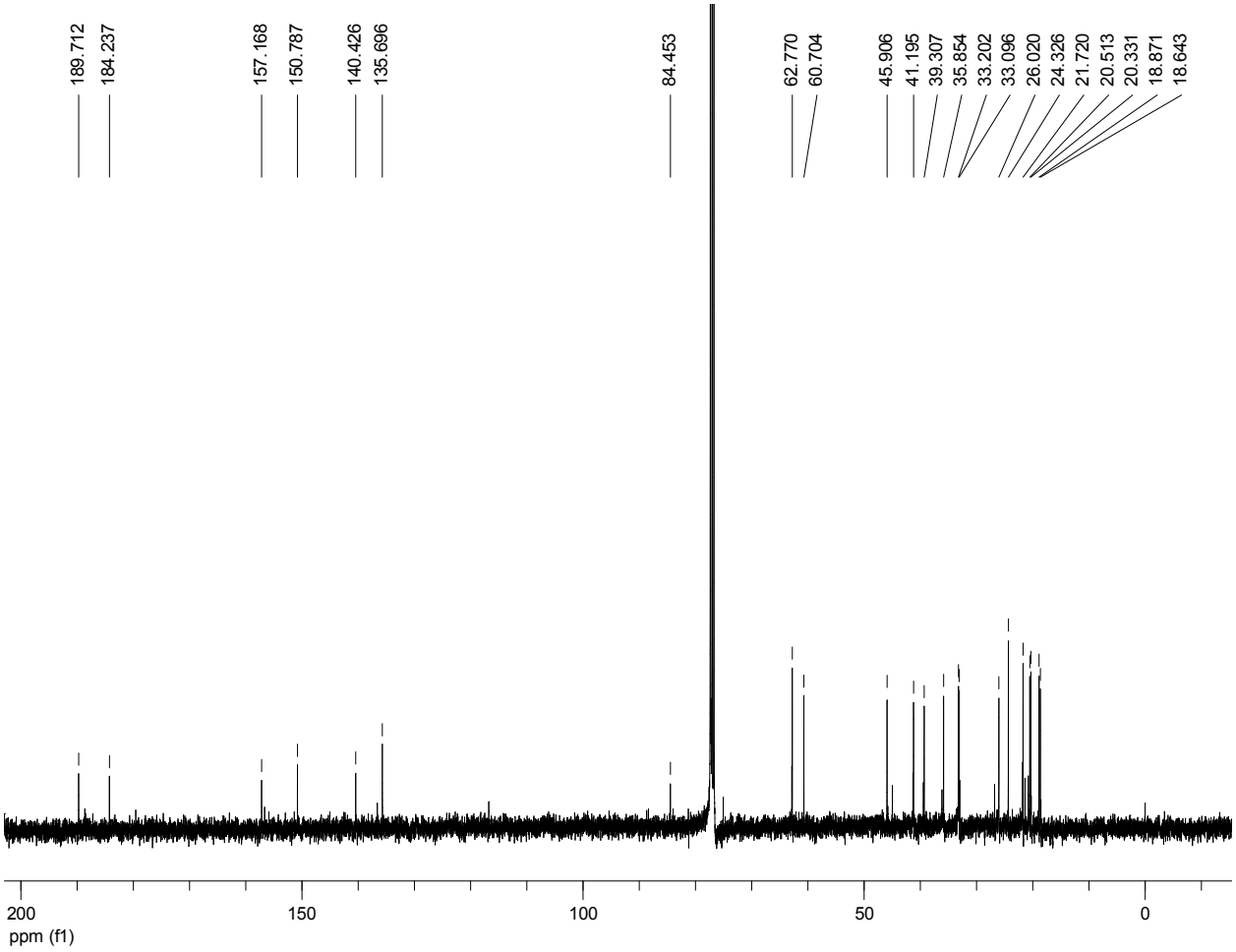
Appendix 1C: ^{13}C-DEPT-135 spectrum of betulinic acid **(39)**



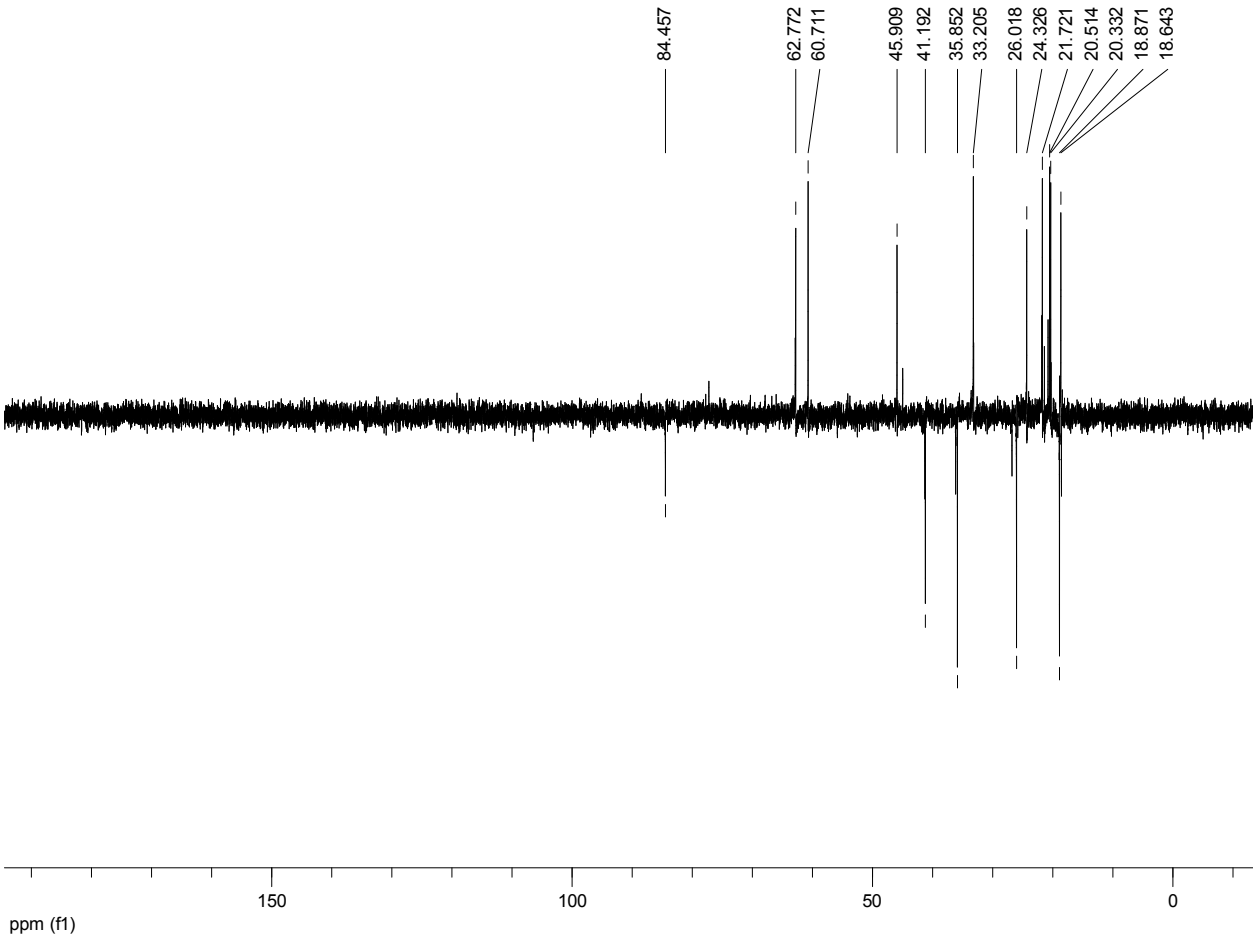
Appendix 2A: ^{1}H-NMR spectrum of compound **40**



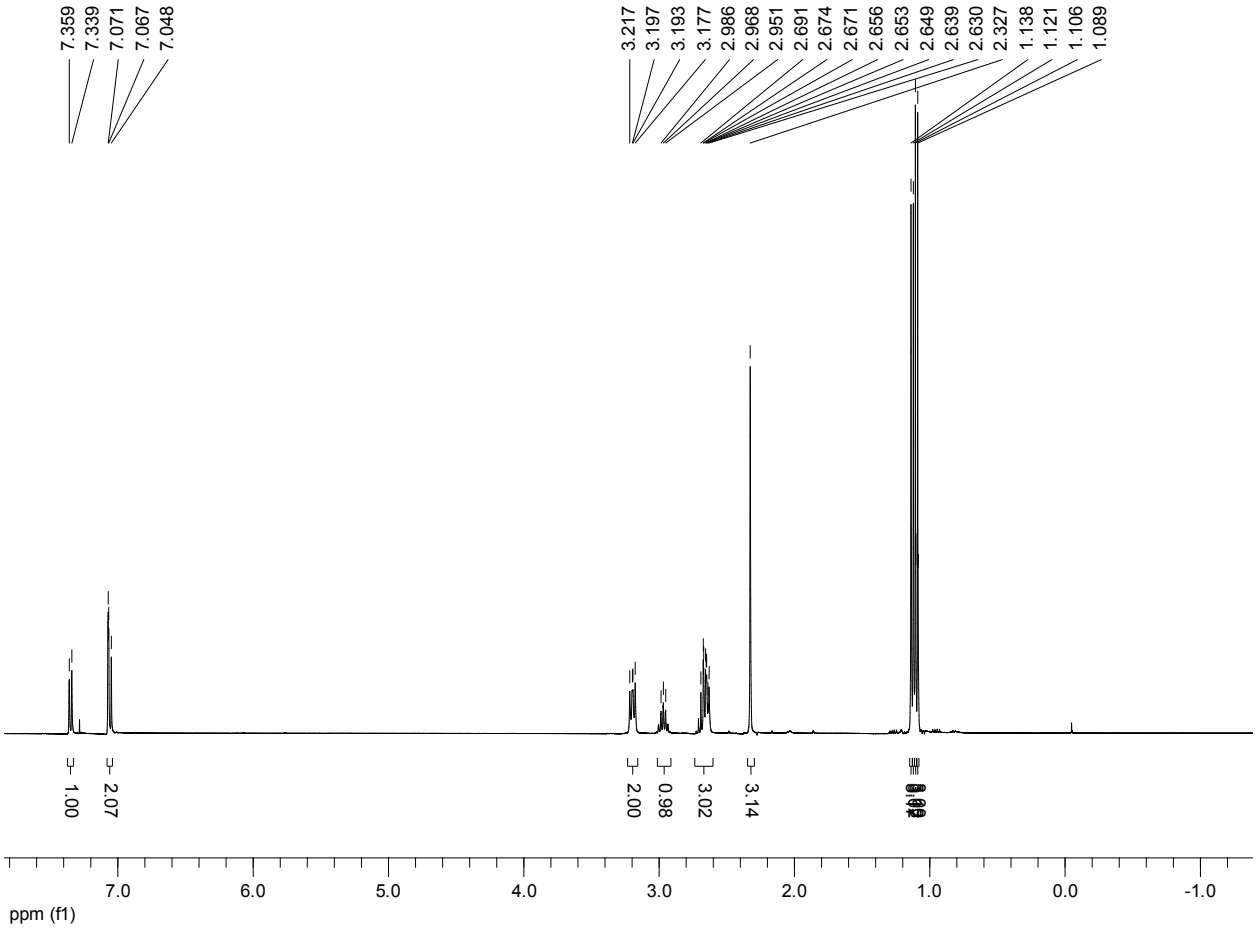
Appendix 2B: ^{13}C-NMR spectrum of compound **40**



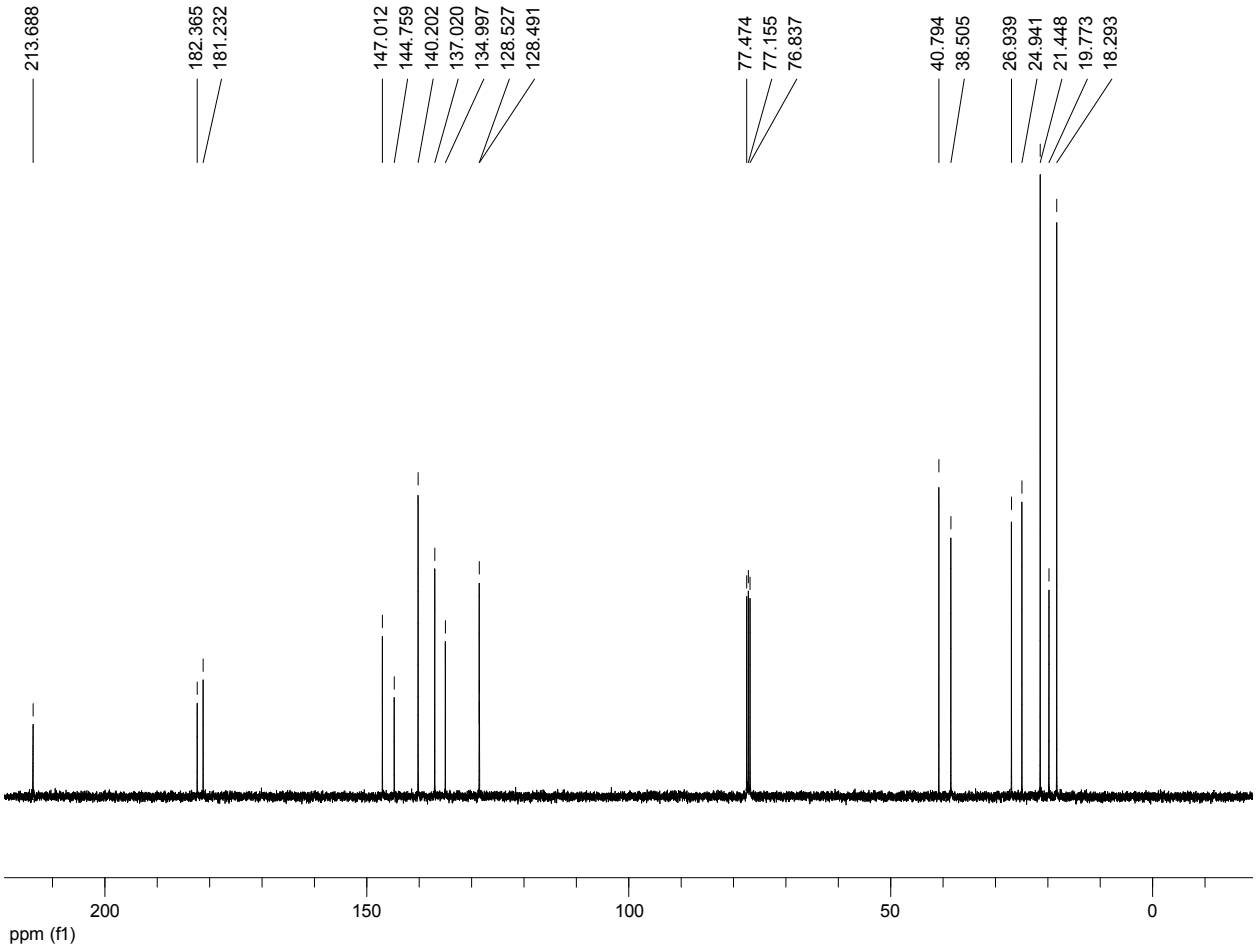
Appendix 2C: ^{13}C-DEPT-135 spectrum of compound **40**



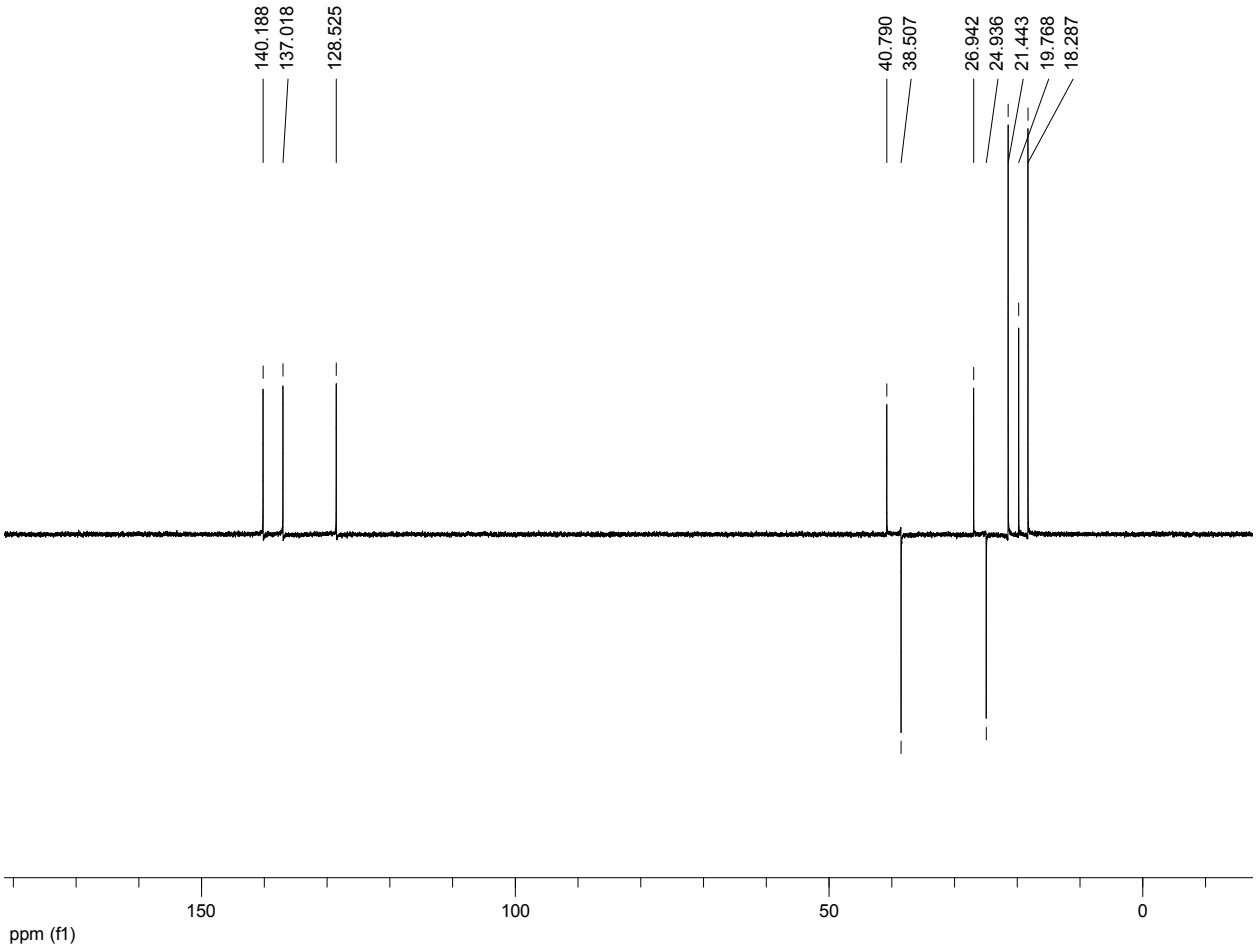
Appendix 3A: ^{1}H-NMR spectrum of compound **41**



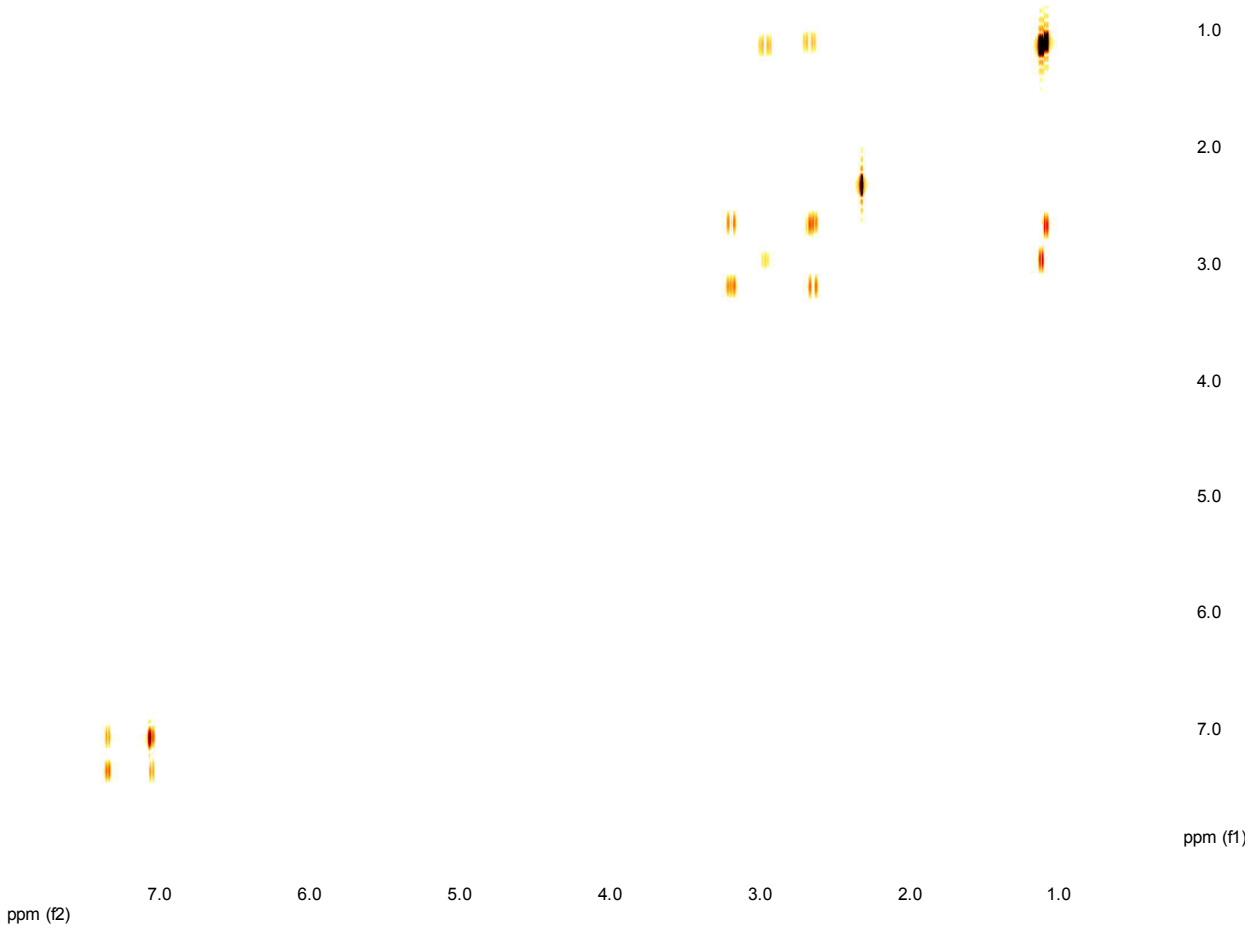
Appendix 3B: ^{13}C-NMR spectrum of compound **41**



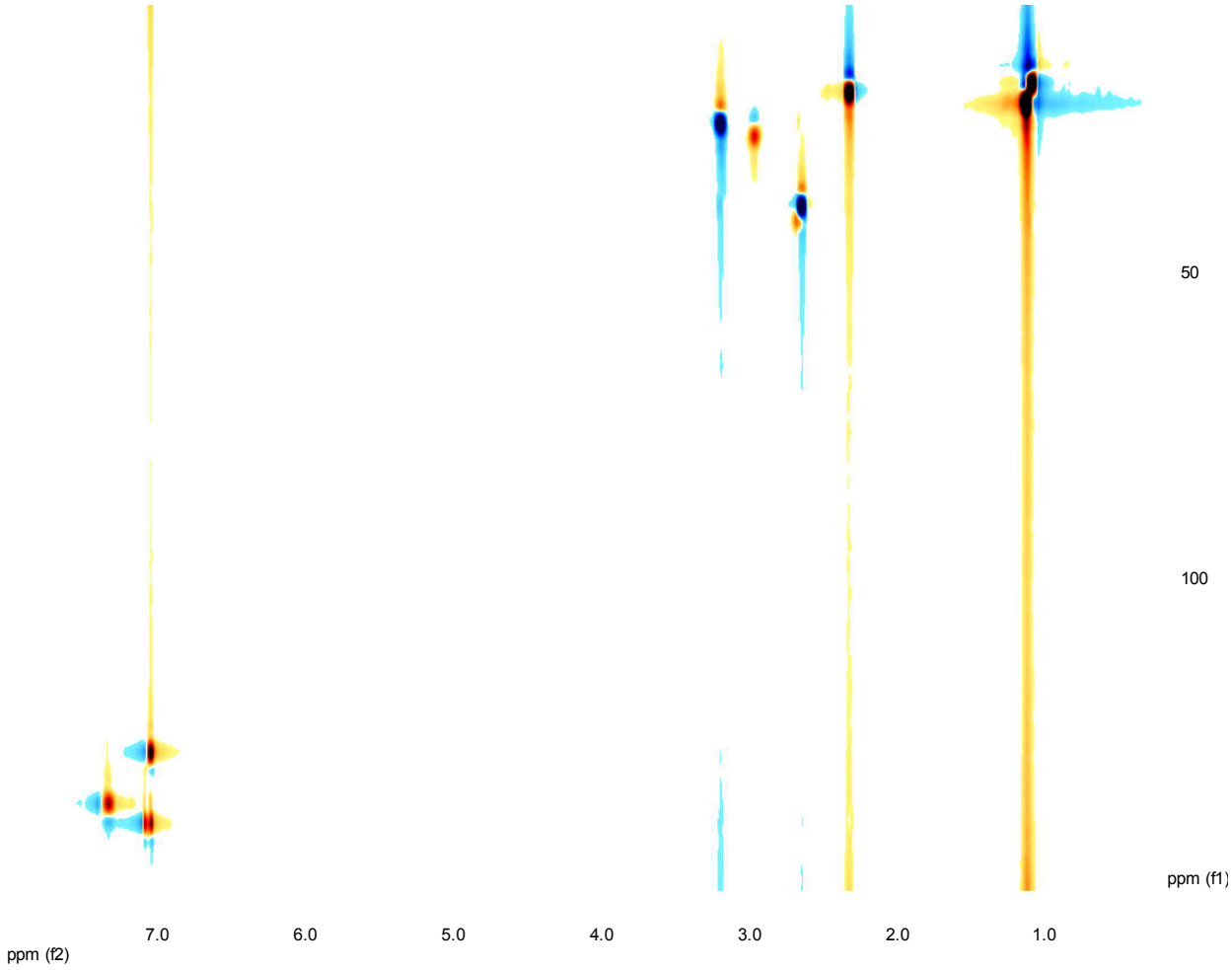
Appendix 3C: ^{13}C-DEPT -135 spectrum of compound **41**



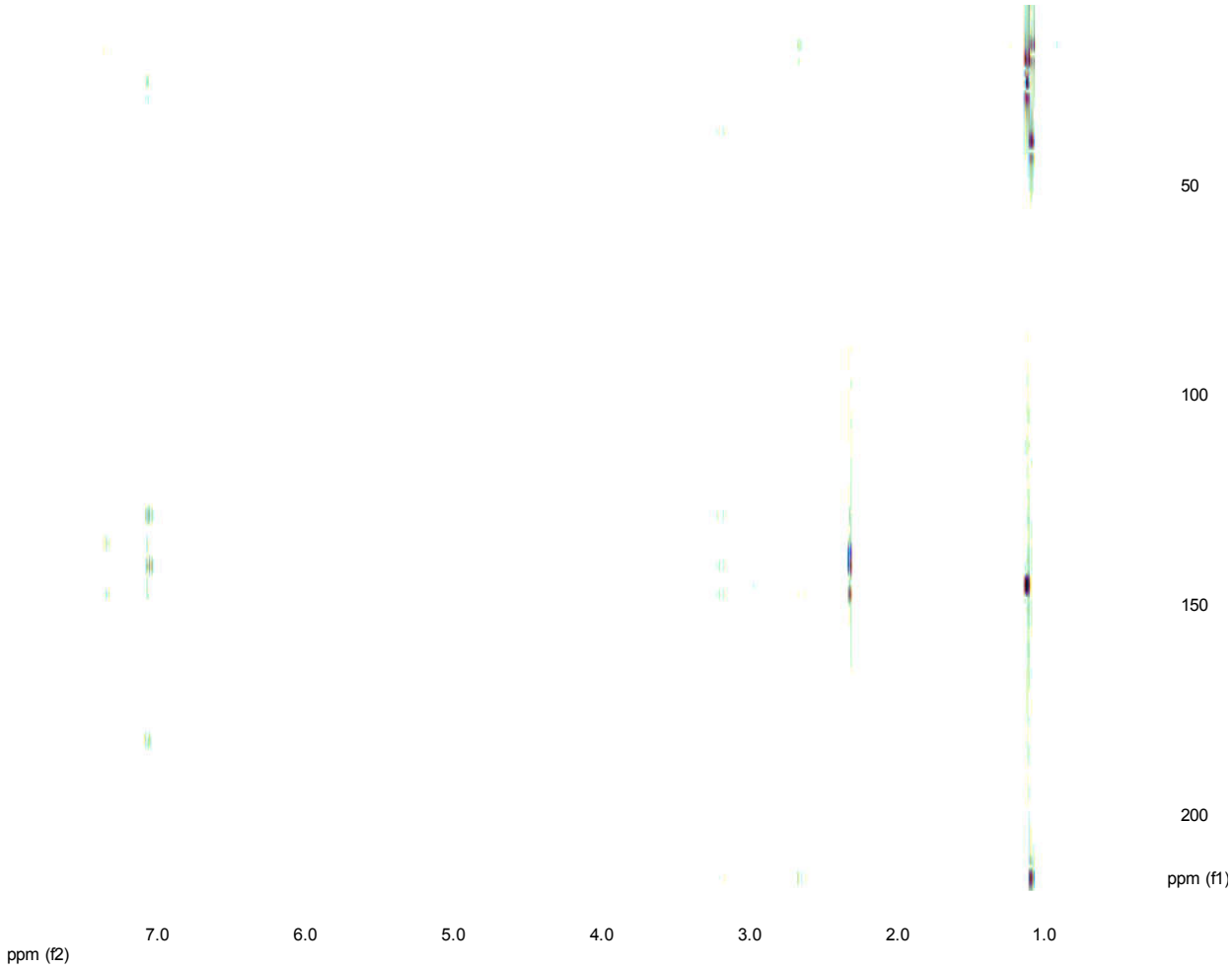
Appendix 3D: 2D-NMR COSY spectrum of compound **41**



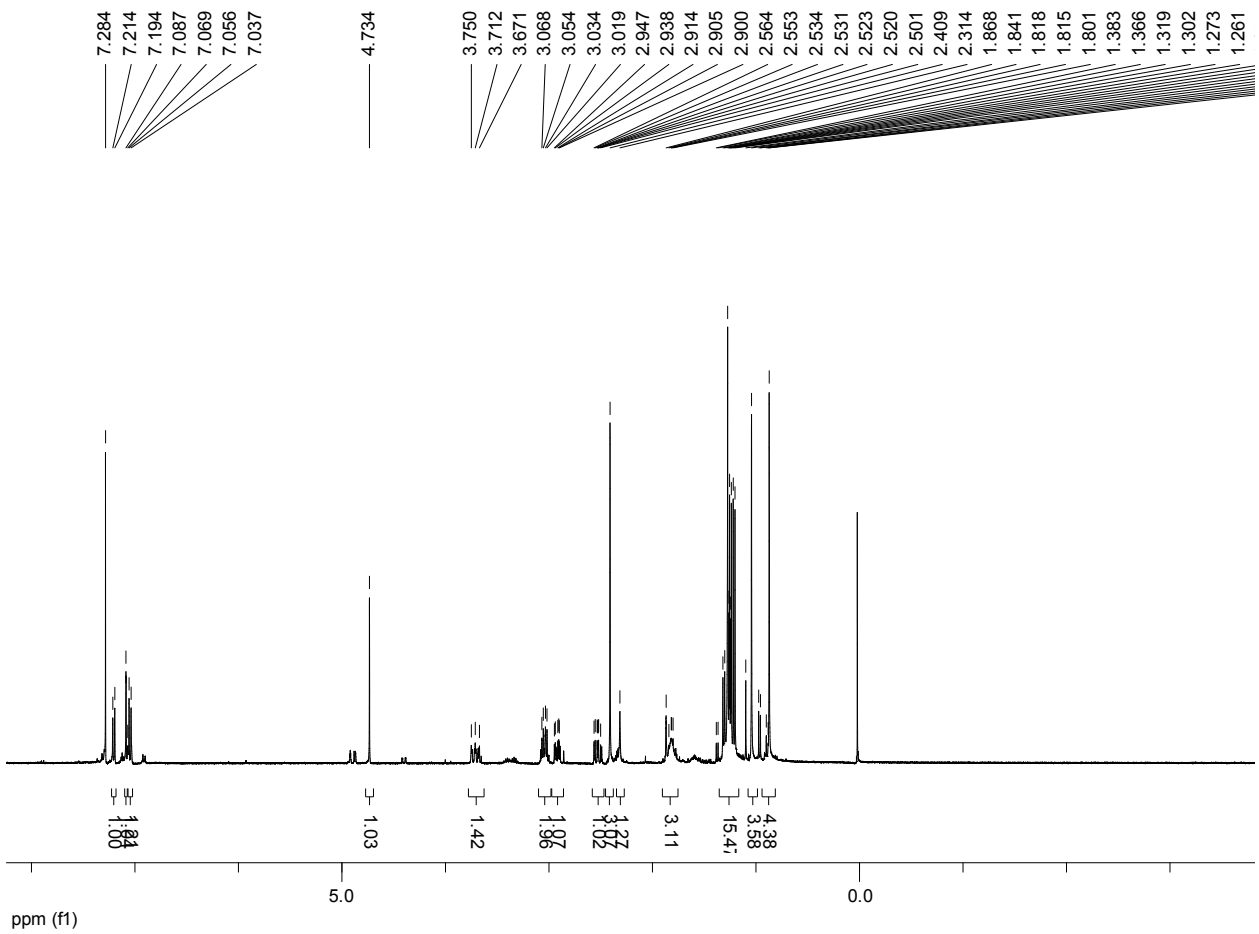
Appendix 3E: 2D-NMR HSQC spectrum of compound **41**



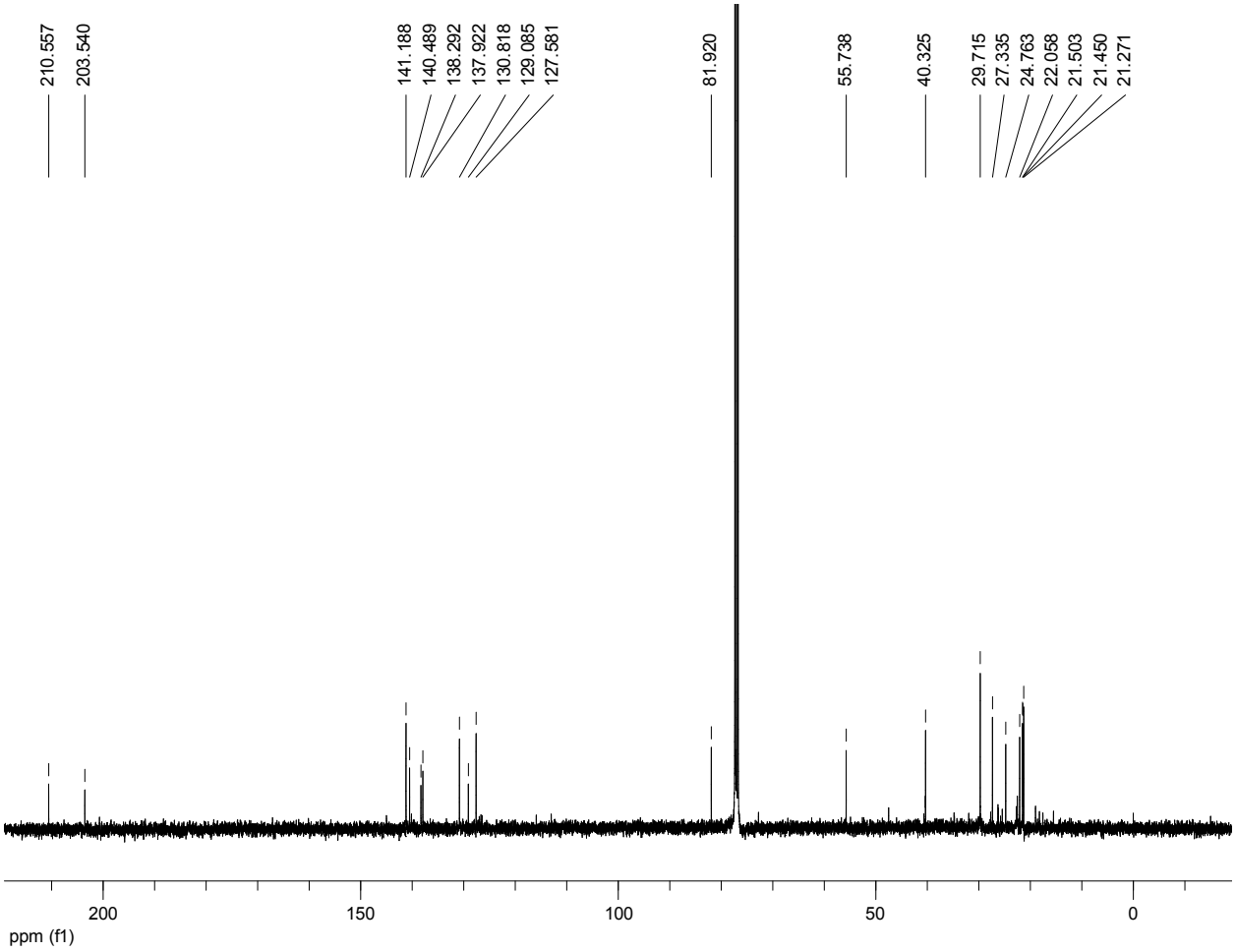
Appendix 3F: 2D-NMR HMBC spectrum of compound **41**



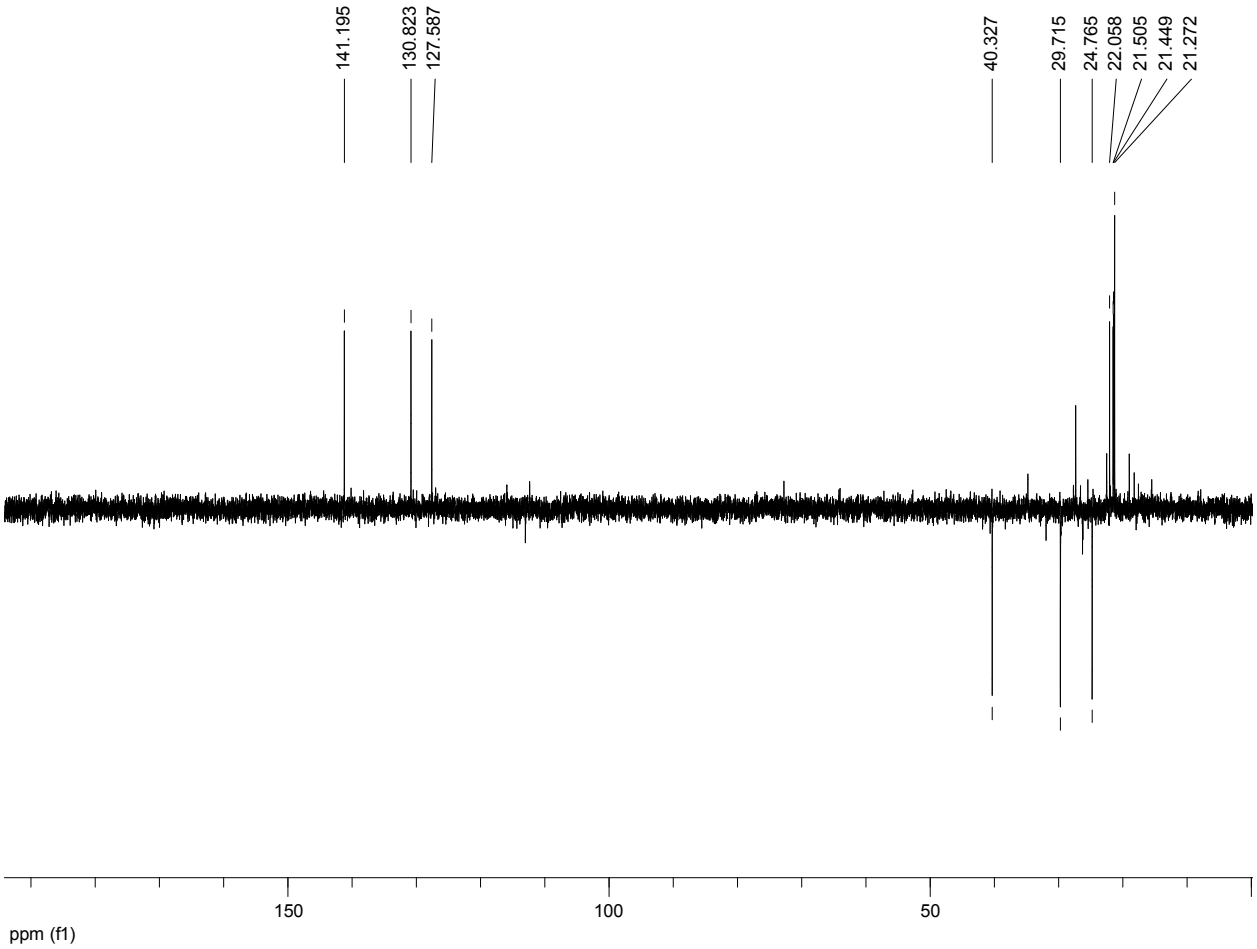
Appendix 4A: ^{1}H-NMR spectrum of compound **42**



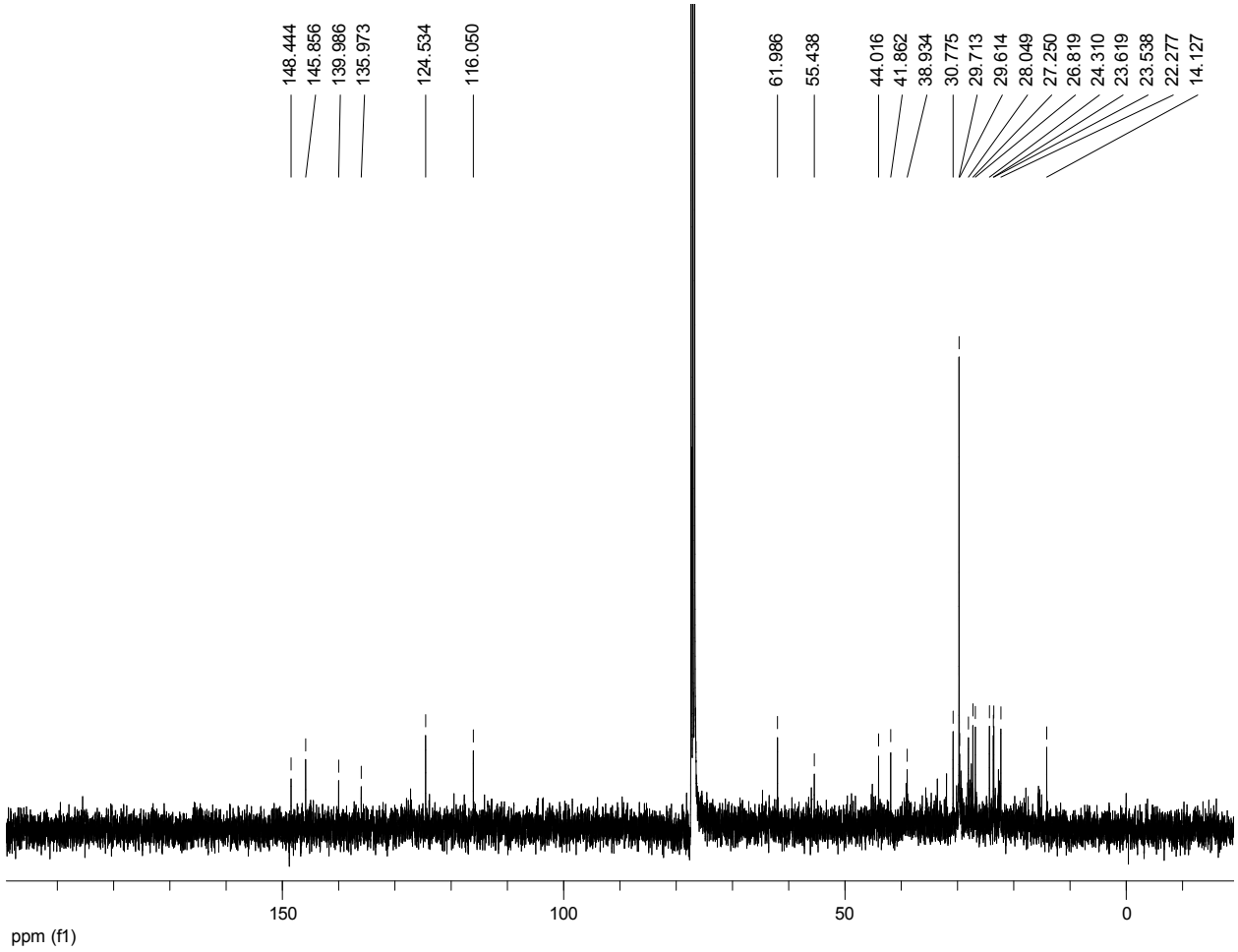
Appendix 4B: ^{13}C-NMR spectrum of compound **42**



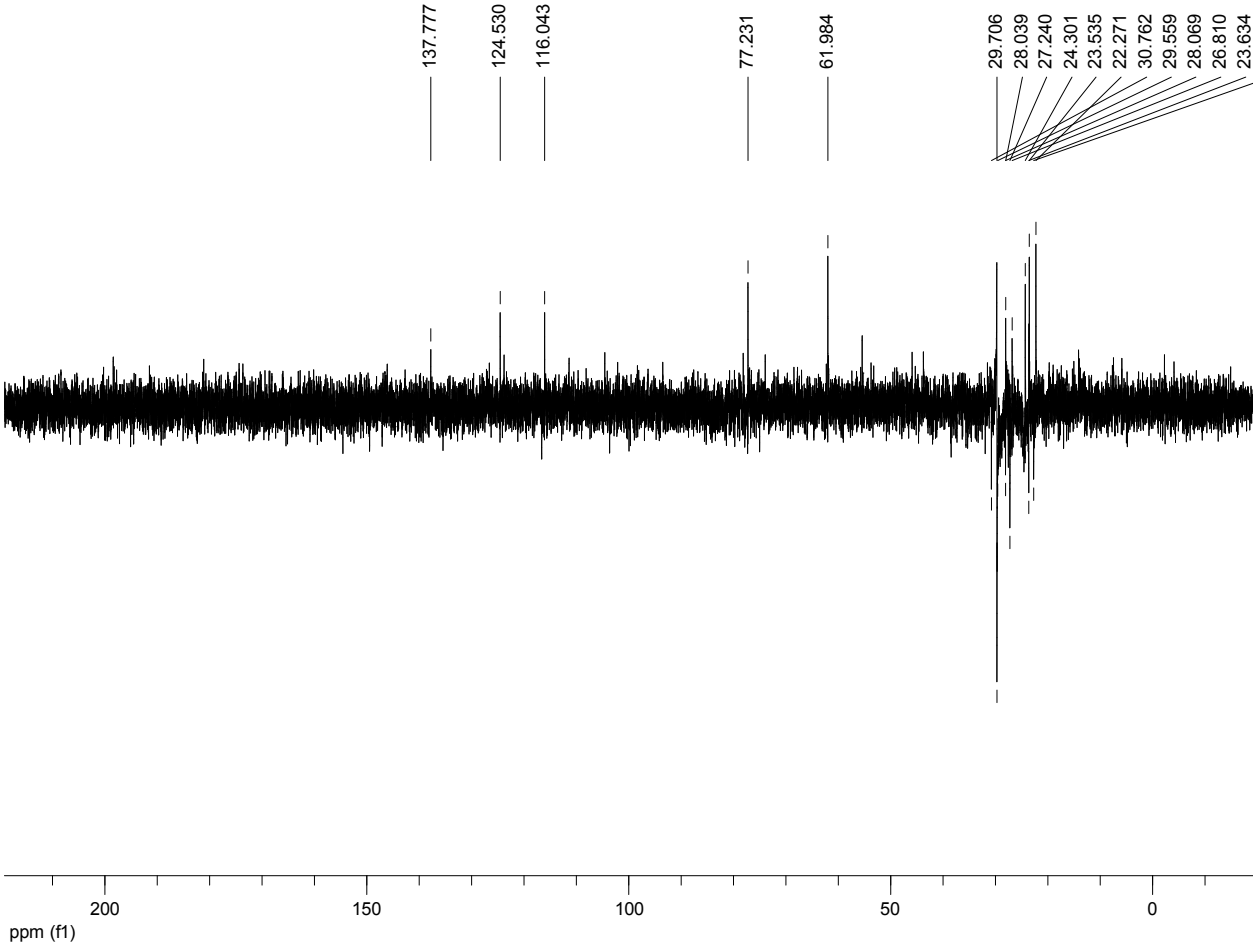
Appendix 4C: ^{13}C-DEPT-135 spectrum of compound **42**



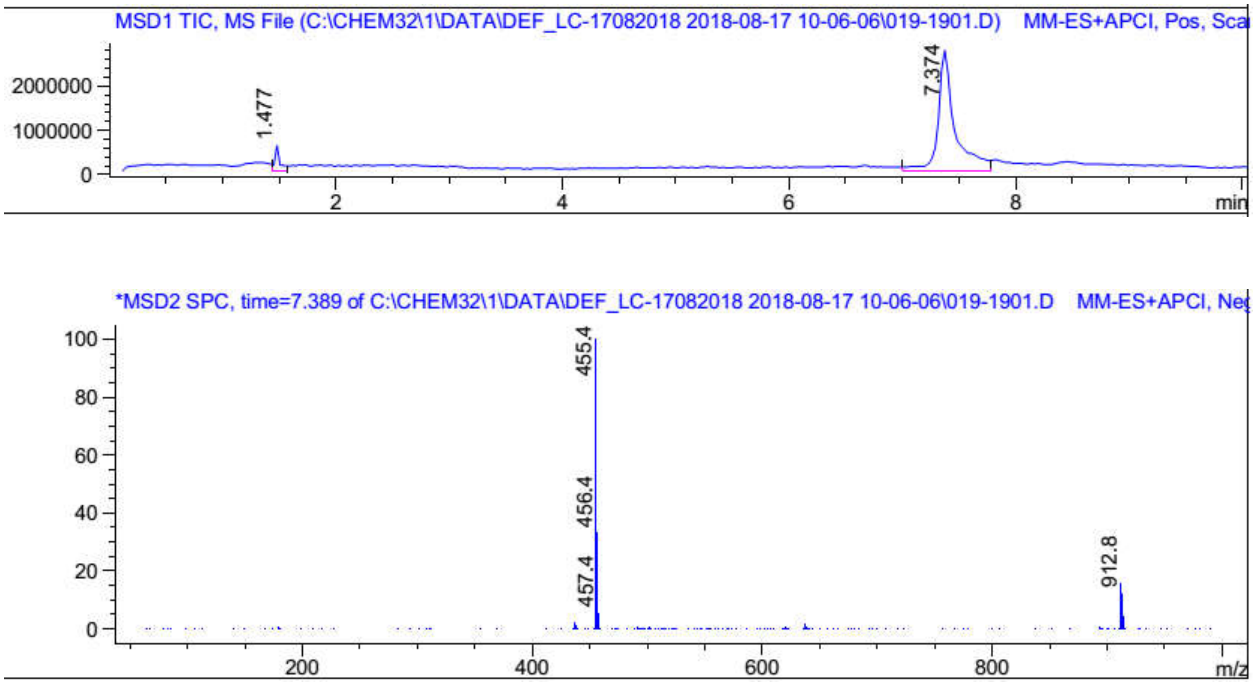
Appendix 5A: ^{13}C-NMR spectrum of compound **43**



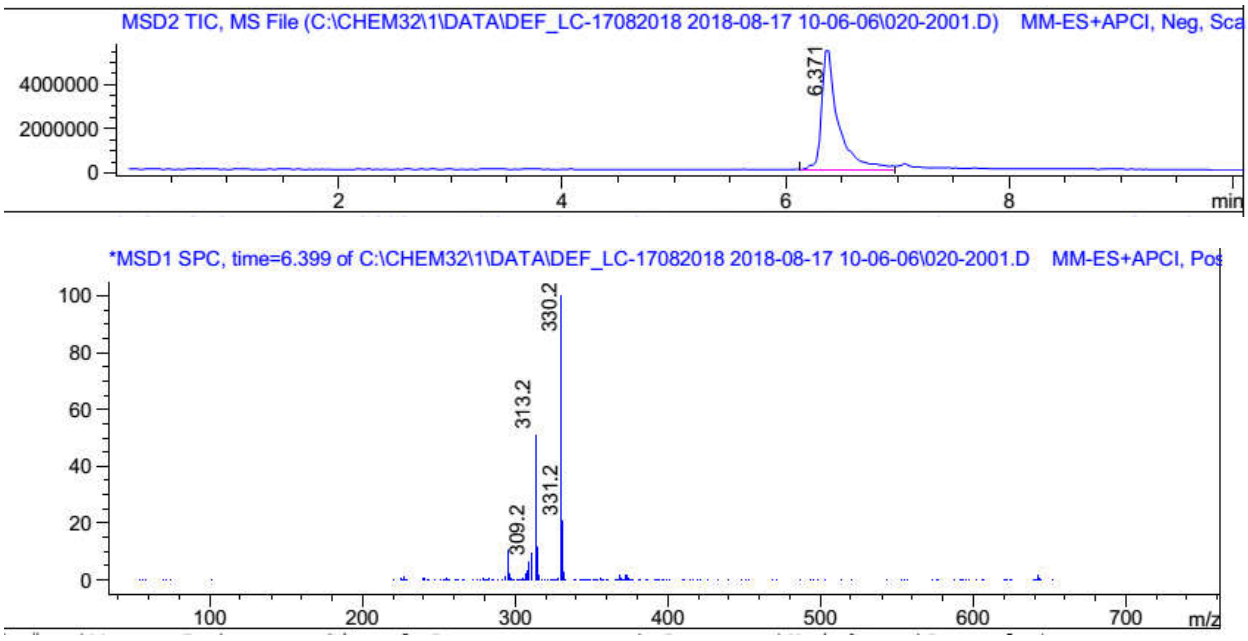
Appendix 5B: ^{13}C-DEPT-135 spectrum of compound **43**



Appendix for ESI-MS and LC chromatographic profile of betulinic acid **(39)**



Appendix for ESI-MS and LC chromatographic profile of Compound **41**



Appendix for ESI-MS and LC chromatographic profile of Compound **42**