PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITIES OF THE SEEDS EXTRACTS AND OILS OF *LEPIDIUM SATIVUM*

BY

DINKAYEHU DESTA

A THESIS SUBMITTED TO

APPLIED CHEMISTRY PROGRAM

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SEPTEMBER, 2017

ADAMA, ETHIOPIA

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ADVISOR: DR. HAILEMICHAEL TESSO (PHD)

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ADVISOR'S APPROVAL SHEET

To: Applied Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled "**PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITIES OF THE SEEDS EXTRACTS AND OILS OF *LEPIDIUM SATIVUM***" submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry, the Graduate program of the Department of Chemistry, and has been carried out by **Mr. Dinkayehu Desta**, **Id. No GSS/0211/05**, under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the Department.

Dr. Hailemichael Tesso (Ph.D) _____ _____

Advisor Signature Date

APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by **Dinkayehu Desta** have read and evaluated his thesis entitled "**PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITIES OF THE SEEDS EXTRACTS AND OILS OF LEPIDIUM SATIVUM**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

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Declaration

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Dinkayehu Desta

Signature: _____

This MSc Thesis has been submitted for examination with my approval as thesis advisor

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Signature: _____

Date of submission: ……..…..

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List of Acronyms

FDA	Food and Drug Administration
UV	Ultraviolet
IR	Infrared
TLC	Thin Layer Chromatography
NMR	Nuclear Magnetic Resonance
^{13}C-NMR	Carbon 13- Nuclear Magnetic Resonance
^{1}H-NMR	Proton-Nuclear Magnetic Resonance
DEPT	Distortion less Enhancement by Polarization Transfer
GC-MS	Gas Chromatography-Mass Spectroscopy
LSM	*Lepidum Sativum* Methanol Extract
LSH	*Lepidum Sativum* n-hexane Extract
LSE	*Lepidum Sativum* Essential oil
FAME	Fatty Acid Methyl Ester
R_f	Retention factor
s	Singlet
d	doublet
t	triplet
Ppm	Parts per million

Abstract

Since long time, the study of organic chemistry contribute to the development of science and better social life by discovering new and useful compounds from nature, especially from plant origin. Objective of the present study was to carry out the phytochemical investigation and antibacterial activities of the seeds extracts and oils of L. sativum obtained from the local area Kersu Kebele, Goma Wereda, Jimma zone.The study was conducted by extraction of the seeds with organic solvents such as n-hexane, dichloromethane, ethyl acetate and methanol. Based on the TLC analysis,the methanol extract was subjected to column chromatography and two compounds coded as ***LSM-9*** *and* ***LSM-11****were isolated and characterized using* ^{13} *C- NMR,* ^{1}*H-NMR, IR and UV-VIs spectroscopic data. In addition, the study performed on extraction of essential oil of the seed through hydrodistillation and investigation of phytochemical constituents of each solvent extract. The n-hexane extract (oil) and the essential oil of the seed extract were analyzed with GC-MS and 11 components were obtained from each types of oil.7,10,13-hexadecatrienoic acid(64.42%) and Indol(63.78%) were the major components of n-hexane extracted and essential oil of the seeds respectively. Moreover both oils were held unsaturated fatty acid, saturated fatty acid and aromatic derivative compounds.The preliminary phytochemical test revealed the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, anthraqunnes, and tannins. Antibacterial activities of the methanol crude extract,* ***LSM-9*** *and the essential oil were implemented by disc diffusion method against one Gram positive bacterium Stphylococus aureus and three Gram negative bacteria: E.coli, Proteus mirabilis and Klibsiella pneumoniae. The inhibition zones of the samples were compared with standard drug ceftriaxone. The essential oil showed antibacterial activities on all the tested bacteria and* ***LSM-9*** *inhibit the tested Gram negative bacteria. While the methanol crude extract did not show inhibition activities against the tested bacteria.*

Key words: *phytochemical investigation, L. sativum, hydrodistillation, extraction, essential oil*

1. INTRODUCTION

1.1. Background of the Study

Organic chemistry as it stands today has developed largely from the chemistry of natural products. The study of natural products has been and continues to be a major deriving force in the development of the fields of organic and medicinal chemistry. Natural products are expected to play an important role as one of the major sources of new drugs in the years to come because of their incomparable structural diversity, the relatively small dimensions of many of them, and their "drug like'' properties, i.e. their ability to be absorbed and metabolized [1]. They are once served man as the source of all drugs, and higher plants provided most of therapeutic agents. Today, 50% of the small pharmaceutically important molecules discovered during the period from 2000 to 2010 have been connected to the field of natural products [2]. Obviously natural products will continue to be extremely important as sources of medicinal agents and biological activities.

The World Health Organization [3] estimates that 80% of the world population relies on local medicinal plants for their primary healthcare needs because of its easy accessibility, affordability and cultural relevance, and about 85% of such medicines involve the use of plant extracts [4]. Therefore, study on natural products is always an interesting target for scientists, especially on plants. Historically, plants (seeds, fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds (natural products). These natural products that are obtained from plants can be basically divided in to two groups with primary and secondary metabolites based on the function in plant metabolism. The primary metabolites of plants constitute of phytochemicals that required for growth such as carbohydrate, amino acids, proteins and chlorophyll. While secondary metabolites consists of alkaloids, saponins, steroids, flavonoids, tannins, glycosides, anthraquienones, terpenoides and others, which are rich in valuable bioactivities, e.g., antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities.

Around the world, the traditional herbal medicines have been widely used for thousands of years. Herbal plants have become the main object of chemists, biochemists, and pharmacists. Their research plays an important role for discovering and developing new drugs, which hopefully are

more effective than and have no side actions like most modern drugs. Thus, such medicinal plants should be investigated to better understand their properties, safety and efficiency [5-7].

In Ethiopia, it has been estimated that traditional remedies are the most important and sometimes the only source of therapeutics for nearly 80% of the population of which 95% of traditional medicinal preparations are of plant origin [8]. Much of the knowledge on traditional medicine is available in rural communities due to inaccessibility of modern healthcare services. Moreover, natural product research is often based on ethnobotanical information and many of the drugs used today were developed from medicinal plants used by indigenous societies [9].

Among the plants used as traditional herbal medicine in Ethiopia and other parts of the world, one is *L. sativum* locally known as "*Fetto*" in Amaharic and "*Cinfa*" in Afan Oromo. It is widely cultivated in Ethiopia parallel with *Teff* (known food crop in Ethiopia) from July to November without irrigation. Due to lack of primary health care facility in the past and the presence of wide variety of vegetation; long time experience of people using traditional medicine in Ethiopia.
Despite the widespread traditional use of *L. sativum* and the presence of many types and sub-class of phytochemical compositions in the plant, little work done inside the country. The insecticidal, repellents and anti-malarial usage of *L. sativum* in Jabitehnan District, West Gojjam has been reported [10], phytochemical screening and antimicrobial activities of crude extract of *L. sativum* seeds grown in Eastern Ethiopia has also been reported [11], and the Genetic Diversity of *L. sativum* populations from Ethiopia reported [12]. *L. sativum* is a useful medicinal plant. However, its phytochemical composition has not been carried out extensively in Ethiopia. Therefore, this study is expected to fill the study gap and increase knowledge on the phytochemical profile of *L. sativum* seed in general.

1.2. Statement of the Problem

Recently, there are some microbial diseases which are multidrug resistance and for which treatment drugs not yet found like different types of cancers and HIV/AIDS. Also, there are drugs associated with side effect that restricts their usage. Moreover, the cost of some drugs is very expensive and difficult to afford by many individuals who have low income. Because of the

mentioned reason above, chemists need to search new and alternative medicines from natural products. As obviously known since long time, natural products of plants are responsible for the approved drugs that are currently available. For example, 18 of the 42 new drugs discovered in 1992 are either natural products or synthetic analog of natural products [13, 14]

L. sativum is an indigenous Ethiopian plant traditionally used for the treatment of various ailments including abdominal pain, skin disease, asthma, cough, eye diseases, malaria, insecticide, insect repellents and given for domestic animals whenever they become sick showing signs such as erect hair and diminished appetite. One Ethiopian's old proverb says that "*Fetto* cure everything''. Therefore, the focus of this study is to isolate compounds from the plant seeds and elucidate their structure by using chromatographic and spectroscopic methods respectively and test the bioactivity of the isolated compounds and essential oils of the seeds against selected microbes.

1.3. Significance of the Study

This study focuses on the phytochemical investigation and antibacterial activities of the extracts of *L. sativum* seed. Isolation and structural elucidation of natural products from *L. sativum* seed have much significances for industrial and pharmacological applications. The study on the extracts of the seeds of *L. sativum* may help to arrive at new natural products. This study may also provide important information about the phytochemical components of *L. sativum's* seed and their structures to the people. Moreover, it can provide initial information for researchers who are interested to conduct further studies on this area.

1.4. Objectives

1.4.1. General Objective

The general objective of this study is to conduct phytochemical investigation and antibacterial activities of the seeds extracts and oils of *L. sativum*.

1.4.2. Specific objectives

1. To extract the dried and powdered seeds of *L. sativum* using n-hexane, dichloromethane, ethyl acetate and methanol successively.
2. To carry out phytochemical screening tests on all of the crude extracts.
3. To extract the essential oil of *L. sativum* seed by hydrodistillation.
4. To analyze the composition of the essential oil and n-hexane extracted seed oil of *L. sativum* by using GC/MS.
5. To isolate secondary metabolites from methanol extract of the seeds of *L. sativum* using column chromatography.
6. To elucidate the structures of isolated compounds using a combination of spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, IR and UV-Vis.
7. To carry out antibacterial activity tests of the essential oil and isolated compounds from solvent extract.

2. LITERATURE REVIEW

2.1. The Family Brassicaceae

Brassicaceae is one of the largest plant families consisting of about 300 genera and 1500 species. It includes vegetable crops, medicinal plants and plants used as food. Several plants of this family are used as antidiabetic, antibacterial, antifungal, anticancer, and show potent insecticidal effects [15-19]. It has a cosmopolitan distribution especially in temperate regions of the north hemisphere. The most common genus of this family are *Anstatica*, *Arabis*, *Diplotaxis*, *Zilla* and *Lepidium* [20,21].

2.2. The Genus *Lepidium*

The genus *Lepidium* is the largest genus of plants in the mustard/cabbage family, Brassicaceae. The genus is widely distributed in the Americas, Africa, Asia, Europe, and Australia, but it is poorly represented in Arctic climates. It includes familiar species such as garden cress, maca and dittander. General common names include pepper cress, pepper grass, and pepper wort. Some species form tumbleweeds. There are about 175 to 220 species in the genus. Some of the species include *L. africanum*, *L. amelum*, *L. arbuscula*, *L. aucheri*, *L. barnebyanum*, *L. banksii*, *L. bipinnatifidum*, *L. biplicatum*, *L. catapycnon*, *L. chichicara*, *L. echinatum*, *L. ecuadoriense*, *L. englerianum*, *L. kalenbornii*, *L. latifolium*, *L. meyenii*, *L. peruvianum*, *L.sativum* [22,23]

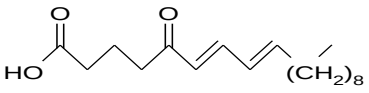
2.3. Taxonomic Classification of *L. sativum* Plant

Taxonomic classification of *L. sativum* plant is:-

Kingdom	=	*Plantae*
Division	=	*Angisospermea*
Class	=	*Dicotyeledonae.*
Subclass	=	*Polypetalae*
Series	=	*Thalamiflorae*
Order	=	*Parietales*
Family	=	*Brassicaceae*
Genus	=	*L. sativum*
Species	=	*L. sativum Linn sp*

2.4. Phytochemistry of the Genus *Lepidium*

The genus *Lepidium* was reported to be rich in secondary metabolites. The phytochemical screening of chloroform, methanol, aqueous and petroleum ether extract of *L. sativum* seed revealed the presence of alkaloids, carbohydrates, phenolic compounds, flavonoids, proteins and amino acids, saponins, mucilage and lipids or fats [24].The alkaloids of *L. sativum* are member of the rare imidazole alkaloids known as Lepidine and Semilepidine [25].The chemical compounds 5-oxo-6E,8E-octadecadienoic acid (**1**), malic acid benzoate (**2**), benzylglucosinolate (glucotropeolin) (**3**), m-methoxybenzylglucosinolate (**4**), benzyl isothiocyanate (**5**), m-methoxybenzyl isothiocyanate(**6**), 5-methylsulfinylpentylglucosinolate (glucoalyssin) (**7**), indolyl-3-methylglucosinolate (glucobrassicin) (**8**), sinigrin (**9**), acaridine (**10**), (1R,3S)-1-methyltetrahydro-β-carboline-3-carboxylic acid (**11**), uridine (**12**), 1,3-dibenzyl-4,5-dimethylimidazolium chloride (lepidiline A) (**13**), 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride (lepidiline B) (**14**), *N*-benzyl-5-oxo-6E,8E-octadecadienamide (**15**) and *N*-benzyl hexadecanamide (**16**) were isolated from the genus *L.* [26,27].



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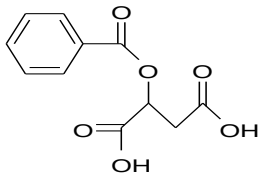

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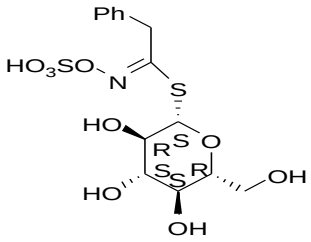

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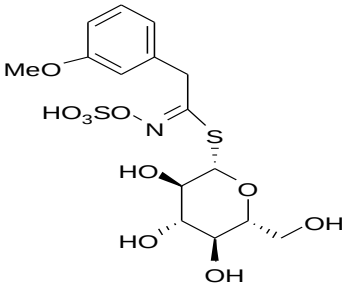

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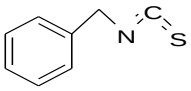

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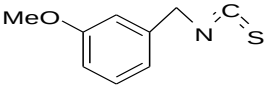

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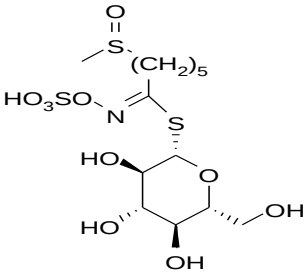

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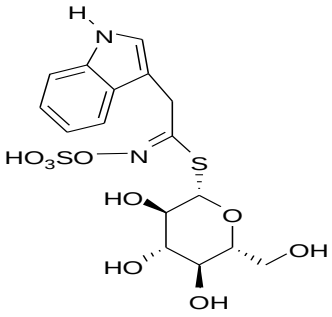

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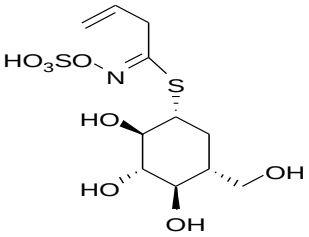

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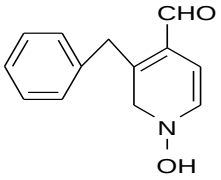

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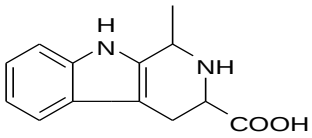

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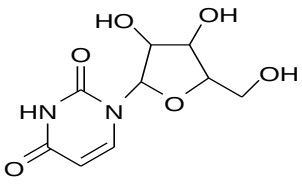

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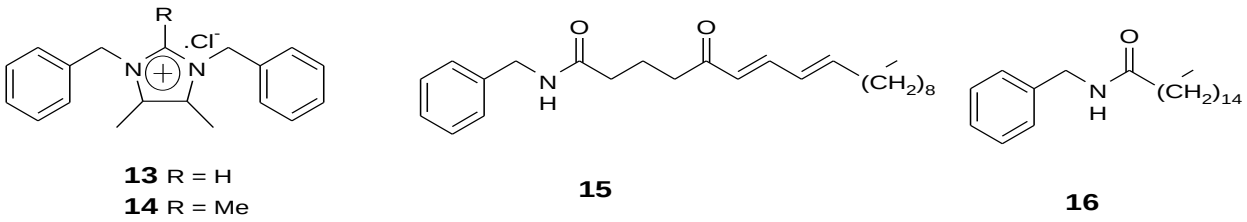


Figure 1: Compounds isolated from the genus *Lepidium.*

2.5. Description of *L. sativum*

L. sativum is an annual herb growing up to 80 cm. Seeds are small, oval-shaped about 2 to 3 mm long and 1 to 1.5 mm wide with reddish brown in color. The stem is finely striate, branched and glabrous (hairless, smooth). In some regions, garden cress is known as garden pepper cress, pepper grass or pepper wort. It is a fast growing edible plant botanically related to water cress and mustard and sharing their peppery, tangy flavor and aroma. The main character of *L. sativum* is that it can grow in any type of climate and soil condition with few requirements. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. It is an important green vegetable consumed by human beings, most typically as a garnish or as a leafy vegetable [28]. Its easy cultivation and its tolerance to different environmental conditions gave it the ability to spread all around the world. About 150 species are found in the temperate and sub temperate areas [29].

Figure 2: Aerial parts (a) and seeds (b) of *L. sativum* (pictures taken by Dinkayehu Desta from Jimma Zone, Goma Wereda, Kersu Kebele)

2.6. Ethnobotanical use of *L. sativum*

L. sativum has been known centuries ago in eastern regions then spread worldwide. Some scientists say its origin is from Ethiopia and then distributed to various parts of the world. Others say that it started from southwest Asia and then spread to Western Europe [30] (Xenophon 400bc). *L. sativum* was famous in Roman and Greek time in their banquets. Mediterranean used it to protect crops from insects and pests [31,32]. In Europe and America, the leaves are used in salad. In various countries of Africa, *L. sativum* seeds are thought to be an effective medicinal remedy to cure respiratory disorders, like bronchitis and asthma [33]. The plant is cultivated as culinary vegetable all over Asia [34]. In south Asia, it is used in traditional medicine to treat stomachic [35].In the countries of Iran and Morocco seeds are used for sexual excitements [36].

2.7. Medicinal Value of *L. sativum*

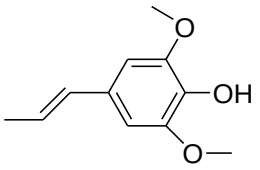
The plant have antiasthmatic, antiscorbutic, aperients, diuretic, stimulant, chemoprotective effect, antidiabetic, antihypertensive, fracture healing properties, hepatoprotective activity, pesticidal activity, antidiarrheal activity [37,38]. Leaf aqueous extracts of *L. sativum* investigated for anticancer activity on human tongue squamous carcinoma (CAL – 27). The results showed that the plant extract inhibit the growth of CAL-27 cells. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactagogue and emmenagogue [39].The root is used in the treatment of secondary syphilis. A preliminary pharmacological study on seeds of *L. sativum* has suggested the presence of cardio active substance and is shown to have probable action through adrenergic mechanisms. The aqueous extract of *L. sativum* seeds has been reported to exhibit a potent hypoglycemic activity. The effectiveness of this plant in treatment of bronchial asthma, hiccups, and cough with expectoration and bleeding piles has been reported. Seeds of *L. sativum* are prescribed by many Ayurvedic practitioners in bronchial asthmatic patients [40]

2.8. Chemical Composition Reported from *L. sativum*

L. sativum is found to contain significant amounts of iron, calcium and folic acid in addition to vitamin A and C. It contains higher amount of protein (25%), glutamic acid (19.3%), and leucine (8.21%). The major secondary compounds of this plant are glucosinolates. On steam distillation, it yields 0.115% of a colorless volatile oil (cress oil) with a characteristic pungent odor. Cress oil

contains variable properties of benzyl isothiocyanate and benzyl cyanide, with a peculiar disagreeable odor [30].

The leaves of *L. sativum* contain protein 5.8%, fats1.0%, carbohydrate 87%, mineral matter 2.2%, calcium 0.36%, phosphorous 0.11%, trace elements iron, nickel and cobalt. Active constituents isolated from *L. sativum* were sinapin (**17**), phenyl acetonitrile (**18**), lepidine (**19**), benzyl isothiocyanate (**20**), sinapic acid (**21**), lepidine A (**22**), lepidine B (**23**), lepidine C (**24**), semilepidine A (**25**), semilepidine B (**26**), hydroxytyrosol acetate (**27**), p-coumaric acid (**28**), protocatechuic acid (**29**), hydroxytyrosol (**30**), p-tyrosol (**31**), dyhydroxymandelic acid (**32**), vainillic acid (**33**) and tetrahydroxymandelic acid (**34**) [27,41].



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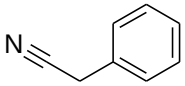

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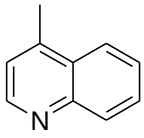

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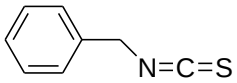

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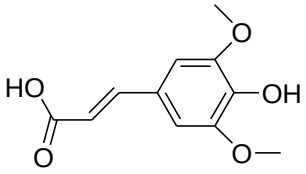

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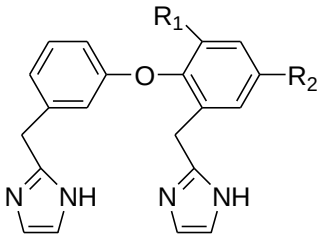
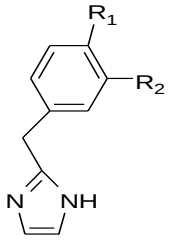
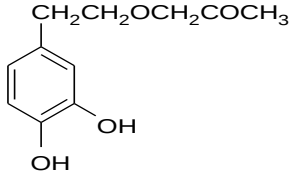


	R1	R2
22	OCH_3	H
23	OH	H
24	H	OCH_3



	R1	R2
25	OH	H
26	OH	OCH_3



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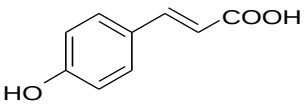

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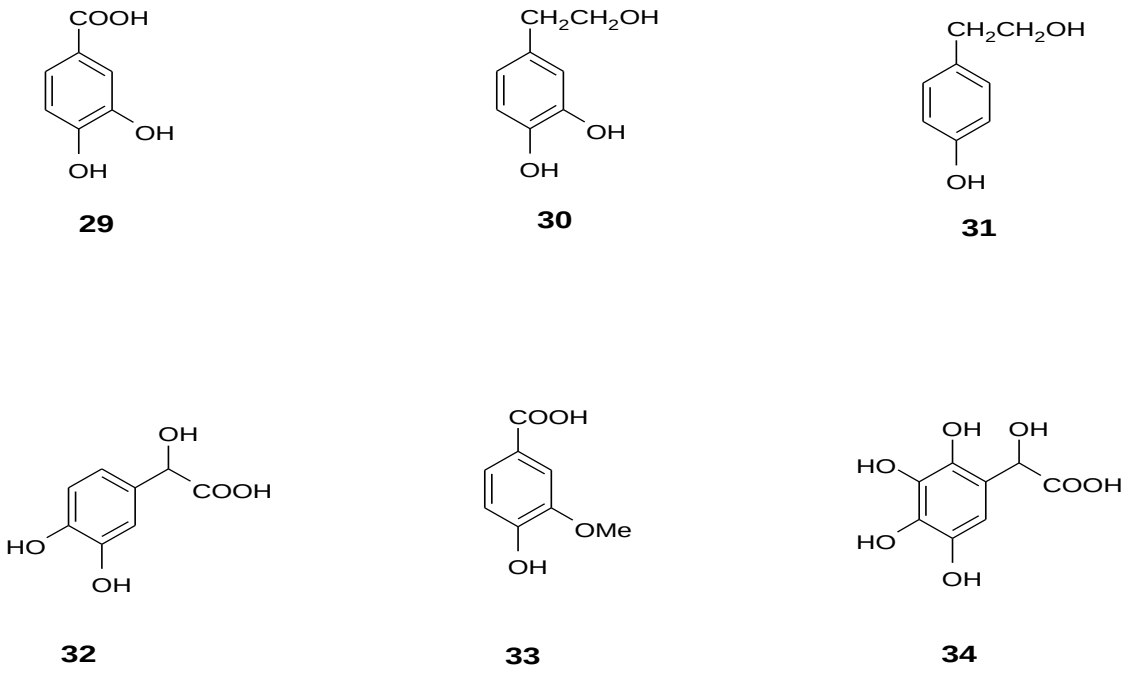


Figure 3: Chemical compounds reported from *L. sativum*

3. MATERIALS AND METHODS

3.1. Plant Material Collection

Seeds of *L. sativum* were collected from Kersu, Goma woreda, Jimma Zone, South west Ethiopia approximately 410 km from Addis Ababa in November 2016. The plant material was authenticated by a taxonomist Melaku Wendafrash and a voucher specimen (Voucher No: 001) was deposited in the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

3.2 Chemicals and Reagents

The study was conducted using the following chemicals: n-hexane (Ranchem industry, India) dichloromethane (Carlo erba reagents, France), ethyl acetate (Atico, India), methanol (Carlo erba reagents, France), chloroform (Blulux Laboratories Pvt. Ltd., India), distilled water, hydrochloric acid, ammonia, ferric chloride, sulfuric acid, Wagner's reagent, silica gel particle size (200-400 mesh).

3.3 Apparatus

The list of apparatus that were used in this research: mortar, pestle, rotary evaporator (model R1001-VNAC 220,50H_Z, China) , round bottom flasks, measuring cylinders, drying oven, filter paper (Whatmann No. 1), separatoy funnels, Water bath, column, Clevenger type apparatus, TLC plate pre-coated (0.2 mm), TLC chamber, iodine, pipettes, UV–lamp, beakers, spatula, conical flasks, vials, refrigerator and tong.

3.4 Instruments

^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker Avance 400 MH_Z spectrometer. IR Spectra was recorded with Perkin Elmer 65FT, scanning range 4000-400cm^{-1}, spectrophotometer using KBr pellets. Ultra-violet and Visible was recorded using T 60 UV-Vis spectrophotometer (200-600 nm) Hitachi U 3200(Japan). GC-MS which is Agilent technologies 7820A GC system with Agilent technologies 5977E MSD, USA. A highly polar capillary column (30 m × 0.25 mm× 0.25 μm film thickness) of HP-88 coated with 100% poly(dimethylsiloxane) was used to separate the FAMEs. Analytical conditions were: injector and detector temperature: 250 and 260°C respectively. The oven temperature was programmed from 60^{o}C to 325/350^{o}C at rate of

6^{o}C/ min. Helium gas was employed as the carrier gas at 1 mL/min flow rate; source 70 eV. Injected volume in the GC-MS was 1μL.

3.5 Experimental Method

3.5.1 Extraction of seed powder of *L. sativum*

The dry plant seed was powdered to suitable size by using mortar and pestle to make it ready for extraction.

Figure 4: powdered seed of *L. sativum*

First 500g of powdered *L. sativum* seed was soaked in n-hexane (2 L) for 72 hrs and shaken occasionally, then, filtered with Whatman No. 1 filter paper and concentrated using rotary evaporator at 40^{o}C to furnish 84.9 g (16.98%) of pale yellow oil and it was kept in refrigerator at 4^{o}C until analysis. The solvent free marc was then soaked in 2 L of dichloromethane for 72 hrs,

filtered, concentrated and 31.2 g (6.24%) of grey yellow oil was obtained. The mark after dichloromethane extract was soaked again in 2 L of ethyl acetate for 72 hrs, filtered and concentrated following the same ways as the previous two extractions and 15.8 g (3.16%) of yellow jelly substance was obtained. Finally, the last marc was soaked for 72 hrs in methanol (2 L), filtered and concentrated using rotary evaporator at 40^{o}C, and afforded 10.2 g (2.04%) of dark reddish jelly crude.

3.5.2 Extraction of essential oil

Dried and pulverized seeds of *L. sativum* (25 g) was suspended in distilled water (350 mL) and placed in a round bottom flask in Clevenger type apparatus and hydrodistilled for 4 hrs. The procedure was repeated four times with new sample to increase the yield and 400 mL hydrodistilled product were collected. Then, the collected amount was divided in to three separatory funnels and mixed with chloroform (equal volume), shaken well and stayed for 24 hrs to clearly separate the organic and water layer.

The organic layer, contained the essential oil separated from water was denser than the water layer and taken position at the bottom of the funnel. Finally, the organic layer was collected in flask. The chloroform evaporated using rotary evaporator at 40^{o}C and 2 g of light red oil obtained. The oil was kept in refrigerator until analysis.

3.5.3 Preparation of fatty acid methyl ester (FAME)

The popular and rapid method for preparation of FAME from oils is base catalyzed trans-methylation by using methanol [42]. This converts the non-volatile fatty acids in the oil into volatile methyl esters that can be easily analyzed by gas chromatography. The trans-esterification was done as described below.

In to a 50 mL bottom flask, one gram of essential oil was weighted and dissolved in 10 ml of 2% methanolic sodium hydroxide solution (prepared by mixing NaOH in methanol) and added a boiling aid. The condenser was fitted to the mixture containing round bottom flask. The content was refluxed at 60^{o}C for 5 min. The mixture was placed in a water bath at 50^{o}C for 1hr reflux, then stopped boiling and removed the condenser, then cooled it to room temperature.The

reaction was stopped by adding small portion of saturated NaCl solution and the solution was swirled gently several times. 20 mL of n-hexane was added to the solution, then the mixture was transferred to the separatory funnel and shaken for a few minutes, then allowed the mixture to settle for some time till it formed layers. The distinct upper layer of methyl ester in n-hexane was separated carefully in a volumetric flask and anhydrous Na_2SO_4 was added to remove any trace of water of the mixture. Then the mixture was filtered with standard Grade 4 qualitative filter paper. Finally, the solvent (n-hexane) was removed from the FAMEs sample using Rota evaporator. The esterified sample was prepared 10ppm in HP capped vial by serial dilution method from 200 ppm stock solution for GC-MS analysis or injection and the components of the oil were identified on the basis of the comparison of retention time and m/z of the compounds with the same compounds found in the NIST 14.L Library.

3.5.4 Preliminary phytochemical screening test on the crude extracts of the seed of *L. sativum*.

The preliminary phytochemical screening test for each of the crude extracts(n-hexane, dichloromethane, ethyl acetate and methanol) of the seeds of *L. sativum* were done according to the method [43,44] to analyze the presence of phytochemical constituents namely: tannins, saponins, alkaloids, flavonoids, glycosides, anthraquinones, and phenols. The results were indicated in **Table 4.**

Test for alkaloids

Wagner's test

Each of the extracts (2 mL) was treated with Wagner's reagents (Iodine and potassium iodide in 100 mL water) and formation of reddish brown precipitate was observed.

Test for flavonoids

Sulfuric acid test

Each of the extracts (2 mL) was treated with concentrated H_2SO_4 and the formation of orange color was observed.

Test for tannins

Ferric chloride test

Each of the extracts (2 mL) was added with alcohol and treated with neutral ferric chloride solution and greenish color was observed.

Test for saponins

Foam test

A small amount of extract was shaken vigorously with water and persistent foam was observed.

Test for anthraquinones

Borntrager's test

To 0.5 g of crude extract, 5 mL of chloroform was added and shaken and then filtered. 0.5 mL of 10% ammonia solution was added to the filtrate and the mixture was shaken well and the violet color was observed.

Test for phenols

Ferric chloride test

Each of the crude extracts (0.5 g) were put in test tube and treated with few drops of 2% of $FeCl_3$ and black color was observed.

Test for glycosides

Borntrager's test

In the first test tube, 1 mL of 5% H_2SO_4 was added to 1mL of the extract solution. The mixture was boiled in a water bath and then filtered to the second test tube. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia and the formation of rose pink to red color was observed.

3.5.5 Isolation of the components from the crude extract

Based on the TLC analysis, the methanol extract was chosen for column chromatography and 6.0 g (adsorbed by equal amount of silica gel) applied on a silica gel column chromatography. The

column was packed with 160 g of silica gel as a stationary phase with 100% dichloromethane. The mobile phase for elution of the column was fixed on the TLC analysis and carried out using the solvent dichloromethane and methanol with increasing polarity begin from 100% dichloromethane and varying the ratio to 90:10, 80:20, 70:30….to 100% methanol. A total of 21 fractions; each of 100 mL were collected.

Table 1: Silica gel column chromatographic fractionation of the MeOH extract

Fraction No	Solvent system	Eluent ratio	Volume collected in mL
1-2	Dichloromethane:methanol	1:0	200
3-6	≫ ≫	9:1	400
7-9	≫ ≫	4:1	300
10-11	≫ ≫	7:3	200
12	≫ ≫	3:2	100
13	≫ ≫	1:1	100
14-16	≫ ≫	2:3	300
17	≫ ≫	3:7	100
18	≫ ≫	1:4	100
19	≫ ≫	1:9	100
20-21	≫ ≫	0:1	200

Fractions showed similar R_f. values: 1- 4, 6-8, 10-12, 13-17 and 18-21 were combined. The spots were visualized by putting the TLC in a material that contain iodine vapor.

For isolating the pure compounds, fractions 6-8, 9, 10-12, 13-17, and 18-21 were separately packed in a column by choosing appropriate solvent system from their TLC analysis. Fraction - 9 was packed in a column chromatography and eluted using the solvent n-hexane:ethyl acetate by increasing polarity and 10 fractions each 10 mL were collected. Fraction seven showed single spot on TLC in iodine vapour and labeled as **LSM-9** (33.5 mg).

Table 2: Rechromatographic fractionation of fraction-9

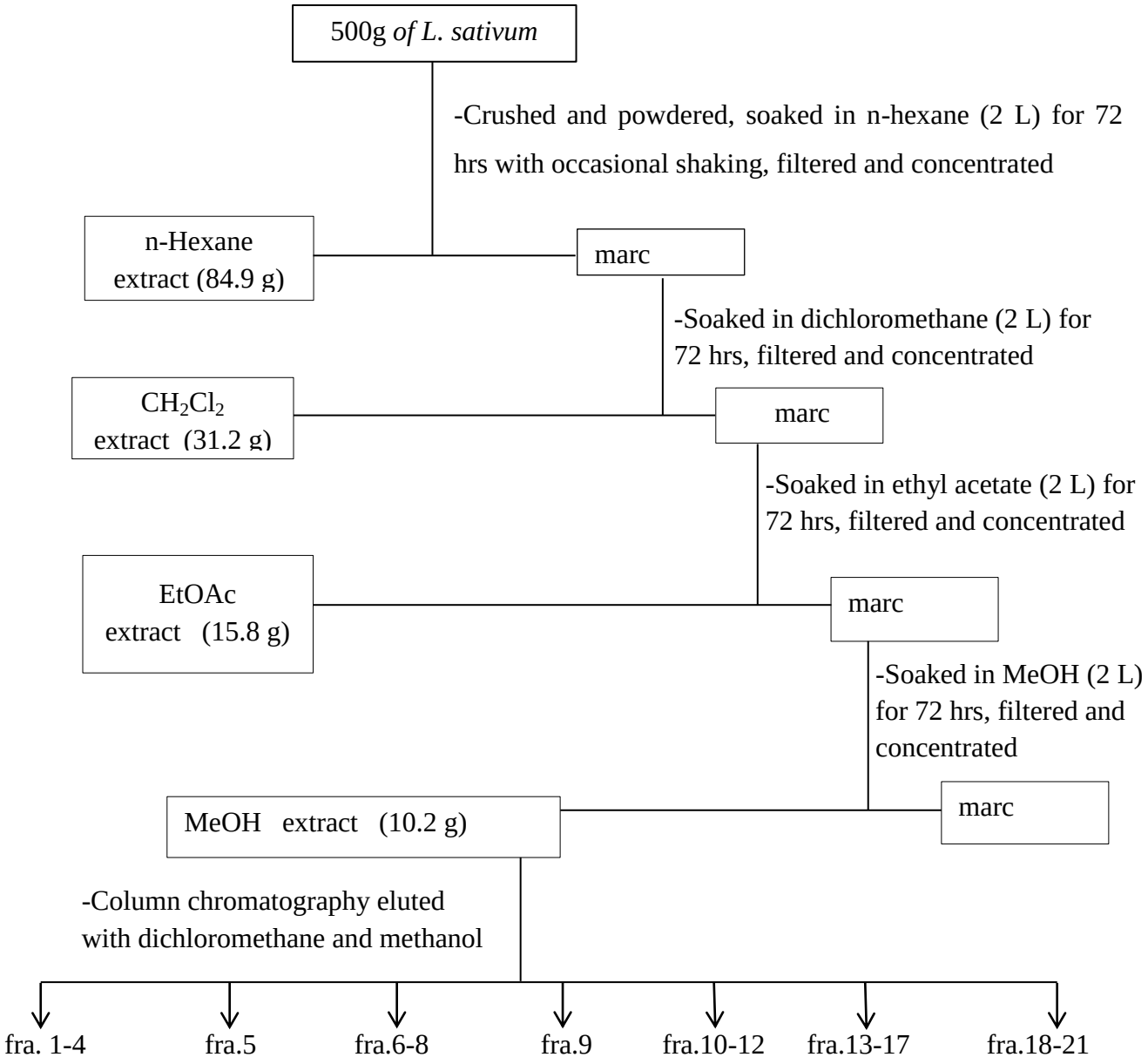
Fraction No	Solvent system	Ratio in mL	Volume collected in mL
1	n-hexane : ethyl acetate	10:0	10
2	≫ ≫	8:2	10
3	≫ ≫	6:4	10
4	≫ ≫	4:6	10
5	≫ ≫	3:7	10
6	≫ ≫	2:8	10
7	≫ ≫	1:9	10
8	≫ ≫	1:9	10
9	≫ ≫	0:10	10
10	≫ ≫	0:10	10

From the silica gel column chromatography of the methanol crude extract, the second compound (white crystal) was obtained by decantation from fractions 10-12 in a solvent system of dichloromethane:methanol in both ratios of 70:30 and 60:40. In fractions of 10-12, first small fine white crystals were observed. Then, collected from each extract through decantation and washed with methanol to purify. weighted to give 40.0 mg and labeled as **LSM-11.**

The isolated 3rd compound (**LSM-15**) was obtained by combining fractions 13-17 and was packed over column chromatography in increasing order with a solvent systems of dichloromethane:methanol. 14 fractions each 10 mL were collected. Fraction 3(26.8 mg) showed single spot on TLC in solvent system dichloromethane:methanol (6:4) as a mobile phase up on dipping in iodine vapour and taken to Addis Ababa University, Ethiopia for NMR analysis. However, the spectral data revealed that **LSM-15** was a mixture of the first two isolated compounds (**LSM-9** and **LSM-11**) and the TLC of this compound was shown below.

Figure 5: The TLC of **LSM-15**

The code was given for the isolated two compounds and two types of oils based on the 1st letters of scientific name of the plant and the 1st letter of extracted solvent. Numbers were also used for the compounds indicated the obtained fractions. All samples were examined by spectroscopic experiments in Addis Ababa University.



Scheme 1: Extraction and isolation of compounds from powdered seeds of *L. sativum.*

3.5.6 Antibacterial activity tests

The methanol crude extract of *L. sativum* seed, the essential oil and compound **LSM-9** were evaluated *in vitro* for antibacterial activity tests by using the disc diffusion method [44]. The antibacterial activities of all samples were tested against four test microorganisms such as one Gram-positive test bacterium *Staphylococcus aureus* and three Gram-negative bacteria: *Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae*. All the microbial were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. The essential oil was used alone, whereas the methanol crude extract (5 mg) and **LSM-9 (**5 mg) were dissolved in 5mL methanol at a concentration of 1 mg/mL. Discs (6mm diameter) were prepared using Whatmman No. 1 filter paper and sterilized by autoclaving at 121^{o}C. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (MHA) surface and impregnated with 20 μL of stock solution. The plates were incubated at 37^{o}C for 24 hrs. The activities of the samples against all bacteria were recorded by measuring the inhibition zone and compared the results with positive control drug for the experiment, ceftriaxone. Zone of inhibition for the standard antibiotic (ceftriaxone) at 20 μL was 23 mm.

4. RESULTS AND DISCUSSION

4.1. Extraction Yields

The solvents that were used during the extraction, the mass of the extracts and percent yield of each of the crude extracts were tabulated in **Table 3.**

Table 3: Percentage yield of solvents extraction

Solvent	Mass of extract obtained in (g)	Percentage yield (%)
n-hexane	84.9 peal yellow oil	16.98
Dichloromethane	31.2 grey oil	6.24
Ethyl acetate	15.8 yellow jelly	3.16
Methanol	10.2 dark reddish jelly	2.04

As shown in **Table 3**, the study result of the solvent extracted seed oil was 23.22%. The yield data also indicated that 15.8 g (3.16%) and 10.2 g (2.04%) were obtained by ethyl acetate and methanol extracts, respectively.

The performed extraction yield of essential oil by hydrodistillation from 100 g of dried seed powder of *L. sativum* was also 2.0 g (2.0%).

4.2. Phytochemical Screening

Results that were obtained from the phytochemical screening tests of n-hexane, dichloromethane, ethyl acetate and methanol crude extracts of *L. sativum* seeds are given in **Table 4** below.

Table 4: Result of phytochemical screening test

No	Constituents	n-hexane	dichloromethane	ethyl acetate	methanol
1	alkaloids	+	+	+	+
2	flavonoids	–	–	–	+
3	tannins	+	+	–	+
4	saponins	+	+	+	+
5	anthraquinones	–	–	+	+
6	phenols	–	+	+	+
7	glycosides	–	–	+	+

Key: + = present - = absent

The result revealed that; alkaloids and saponins were found in all solvent extracts and flavonoids did not show positive test in n-hexane, dichloromethane and ethyl acetate extracts. More phytochemical constituents were obtained in the methanol crude extract when compared with other crude extracts and this implemented that, the seeds of *L. sativum* held polar compounds.

4. 3. TLC analysis of the crude extracts

The TLC profiles for each of the crude extracts of the seeds of *L. sativum* are shown in **Figure 6** below.

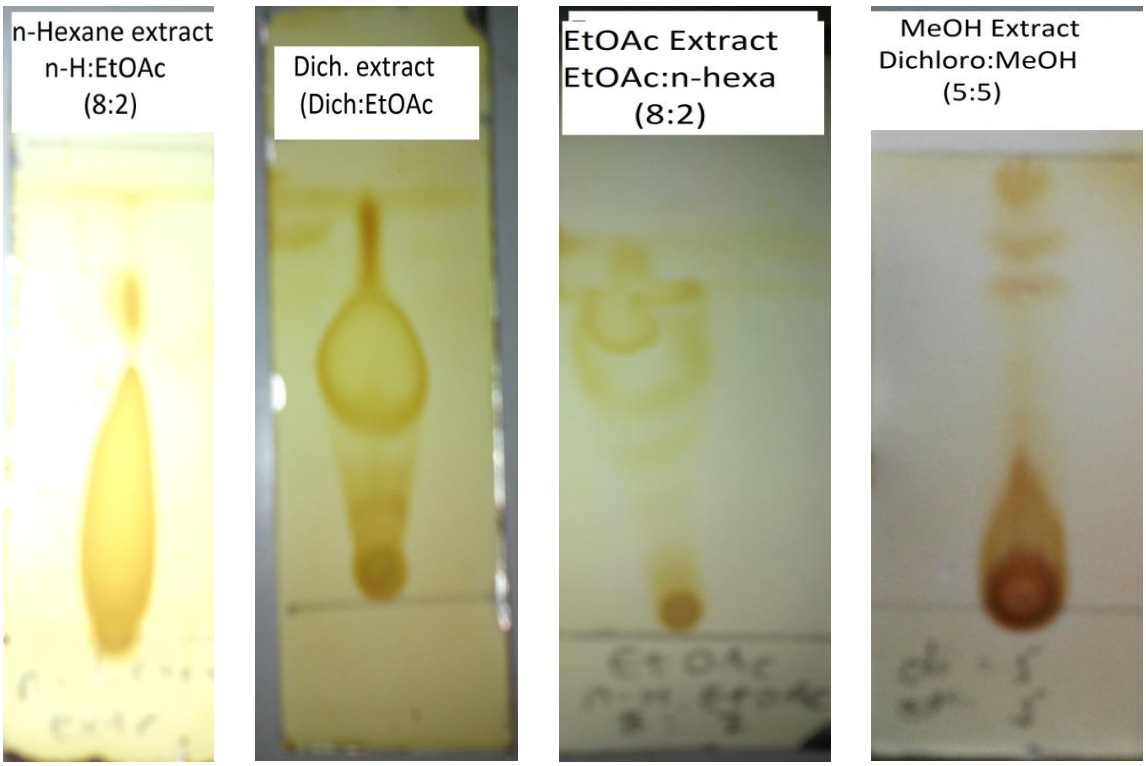


Figure 6: The TLC profile of crude extracts

As shown above in **Figure 6**, the TLC profile of the methanol crude extract showed more than four spots (**Figure 6d**) with different R_f. values in dichloromethane:methanol (1:1) solvent system as a mobile phase. The TLC analysis of other crude extracts did not show promising spots. Therefore, the methanol crude extracts were subjected to silica gel column chromatography.

4.4. Isolation of Compounds

In this study, two compounds were isolated. Compound one named as **LSM-9** (33.5 mg) was isolated from fraction - 9 by repeated silica gel column chromatography with a solvent system of

n-hexane:ethyl acetate (1:9). Compound -2 named as **LSM-11**(40.0 mg) was a white crystal obtained from fraction 10-12 with a solvent system of dichloromethane:methanol (70:30) and (60:40).

4.5. Characterization of Isolated Compounds.

4.5.1 Characterization of LSM-9

The compound **LSM-9** (33.5 mg) was dark reddish jelly, isolated from fraction - 9 with a solvent system of n-hexane:ethyl acetate (1: 9) with $R_{f.}$ value of 0.6.

Figure 7: TLC profile of LSM-9

Structural elucidation of the compound was carried out based on the spectroscopic data obtained from NMR (^{1}H-NMR, ^{13}C- NMR and DEPT-135), IR, UV-Vis spectra. The sample was dissolved in DMSO for NMR spectral analysis.

The UV-Vis. spectrum of compound **LSM-9** showed absorption maxima at 275 and 344 nm indicating the presence of conjugation in the structure of the molecule.

The IR spectral data of the compound showed strong and broad absorption band around 3383 cm^{-1} indicated the presence of hydrogen bonded -OH stretching. The absorption band at 2912 cm^{-1} indicated the presence of aliphatic C-H bond stretching. The band at 1457 cm^{-1} indicated CH_2 deformation. While the bands at 1599 cm^{-1},1263 cm^{-1} and 1060 cm^{-1} indicated the presence of C=C, C-O, and S=O, bond stretching, respectively.

The ^{1}H-NMR spectrum showed five aromatic protons at δ 7.25, 7.34, 7.17, 7.34, and 7.25, five CH (methine) protons at δ4.34, 3.72, 3.43, 3.05, 3.90 and two methylene protons at δ2.98 and (4.13,4.16) (**Table 5**).

The data obtained from ^{13}C-NMR spectrum revealed that the compound **LSM-9** contained 14 carbon atoms. The aromatic carbons appeared at δ137.0, 129.0, 128.5 and 127.3 and aliphatic carbons at δ38.2, 155.9, 81.7, 73.2, 78.5, 70.2, 81.6 and 61.4. Comparison of ^{13}C-NMR spectra with DEPT-135 spectra showed that the peak at δ137.0 and 155.9 were quaternary carbons and the peaks at δ38.2 and 61.4 showed down ward signals in the DEPT-135 indicating two CH_2 (methylene) carbons. The peaks at δ129.0, 128.5, 127.3, 81.7, 73.2, 78.5, 70.2 and 81.6 were indicated the CH (methine) carbons (**Table 5**). NMR spectral data listed above are in a good agreement with the compound benzylglucosinolate. Benzylglucosinolate is a secondary metabolite found mainly in Brassicacea.The compound is hydrolyzed by the enzyme myrosinase when plants are stressed by biotic and abiotic factors, obtaining different degradation products such as isothiocyanate, nitrile and thiocyanate [46]. Benzylglucosinolate and its derivatives involved in plant defense reactions and have benficial effects on human health, because they are strong antioxidants [47]. Several studies have strongly suggested that, benzylglucosinolate help a decrease the risk of certain cancers as well a reduced risk of several diseases, such as diabetes, cardiovascular alterations and bacterial infections due to the oxidative stress[48].

Table 5: ^{1}H-NMR, ^{13}C- NMR and DEPT-135 spectral data of **LSM-9**

Carbon position	^{1}H-NMR δ(ppm)	Remark	^{13}C- NMR δ (ppm)	DEPT- 135	Remark
1	_	_	137.0	_	C (quaternary)
2	7.25	1H, *d*, *J* = 4.4 Hz	129.0	129.0	CH
3	7.34	1H, *t*, *J* = 6.8 Hz	128.5	128.5	CH
4	7.17	1H, *t*, *J* = 5.2 Hz	127.3	127.3	CH
5	7.34	1H, *t*, *J* = 6.8 Hz	128.5	128.5	CH
6	7.25	1H, *d*, *J* = 4.4 Hz	129.0	129.0	CH
1’	2.98	2H, *s*	38.2	38.2	CH_2
2’	_	_	155.9	_	C (quaternary)
1’’	4.34	1H, *d*, *J* = 9.2 Hz	81.7	81.7	CH
2’’	3.72	1H, *t*, *J* = 11.2 Hz	73.2	73.2	CH
3’’	3.43	1H, *t*, *J* =6.8 Hz	78.5	78.5	CH
4’’	3.05	1H, *t*, *J* = 8.0 Hz	70.2	70.2	CH
5’’	3.90	1H, *m*	81.6	81.6	CH
6’’	4.13 and 4.16	1H, *d*, *J* = 13.2 Hz and 1H, d, *J* = 12.4Hz	61.4	61.4	CH_2

Based on the spectral data, the proposed compound has the following structure.

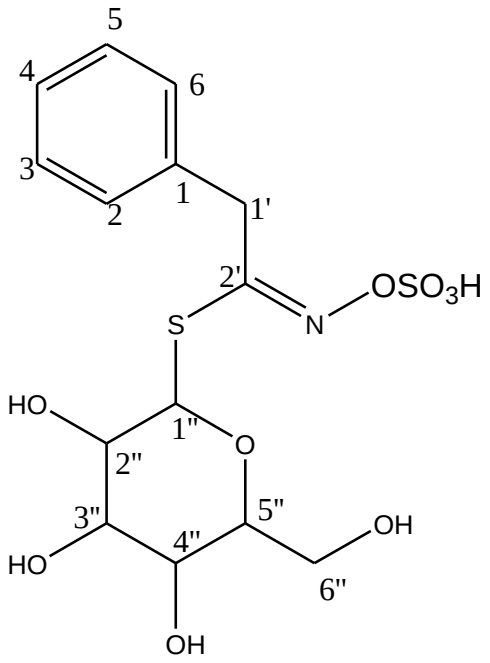


Figure 8: The proposed structure of **LSM-9**

4.5.2 Characterization of LSM-11

The compound **LSM-11**(40.0 mg) isolated as a white crystal solid with a solvent system of dichloromethane: methanol (70:30) and (60:40). The structure was elucidated based on the data obtained from NMR (^{1}H- NMR, ^{13}C- NMR and DEPT-135) and IR, spectra. The sample was dissolved in D_2O for the NMR spectral analysis.

The IR spectra of the compound showed absorption peak at 3442 cm^{-1} due to the -OH stretching and absorption peak at 2925 cm^{-1} due to the C-H stretching. The peak at 1435 cm^{-1} due to CH_2 deformation. While the peaks at 1211 cm^{-1} and 1040 cm^{-1} due to C-O stretching.

The ^{1}H-NMR spectral data, indicated eight aliphatic methine protons at δ 5.30, 3.45, 3.64, 3.35, 3.74, 4.09, 3.93, 3.77, and three methylene protons at δ 3.70, 3.59, and 3.70 (**Table 6**).

From the ^{13}C- NMR spectra, the compound **LSM-11** contained 12 aliphatic carbons at δ92.1, 71.0, 72.5, 69.2, 72.4, 60.1, 61.3, 103.6, 76.4, 74.0, 81.3 and 62.3. Comparing the ^{13}C- NMR spectra from DEPT-135 spectra, the peaks at δ62.3, 61.3 and 60.1 showed down ward in the DEPT were CH_2 (methylene) carbons. The eight carbon signals were appeared at δ92.1, 71.0, 72.5, 69.2, 72.4, 76.4, 74.0, 81.3 revealed CH (methine) carbons and the observed peak at δ103.6 indicated one quaternary carbon (**Table 6**). The spectral analysis of **LSM-11** was found in a good agreement with the compound sucrose.

Table 6: ^{1}H- NMR, ^{13}C- NMR and DEPT-135 spectral data of **LSM-11**

Carbon position	^{1}H-NMR δ (ppm)	Remark	^{13}C- NMR δ (ppm)	DEPT-135	Remark
1	5.30	1H, *d*, *J* = 4.0 Hz	92.1	92.1	CH
2	3.45	1H, *dd*, J_1=4.0, J_2=4.0	71.0	71.0	CH
3	3.64	1H, *t*, *J* = 9.6 Hz	72.5	72.5	CH
4	3.35	1H, *t*, *J* = 9.2 Hz	69.2	69.2	CH
5	3.74	1H, *m*	72.4	72.4	CH
6	3.70	2H, *d*, *J* = 2.4 Hz	60.1	60.1	CH_2
1’	3.59	2H, *s*	61.3	61.3	CH_2
2’	_	_	103.6	_	C (quaternary)
3’	4.09	1H, *d*, *J* = 8.4 Hz	76.4	76.4	CH
4’	3.93	1H, *t*, *J* = 8.4 Hz	74.0	74.0	CH
5’	3.77	1H, *m*	81.3	81.3	CH
6’	3.71	2H, *d*, *J* = 2.4 Hz	62.3	62.3	CH_2

Based on the spectral data, the proposed compound has the following structure.

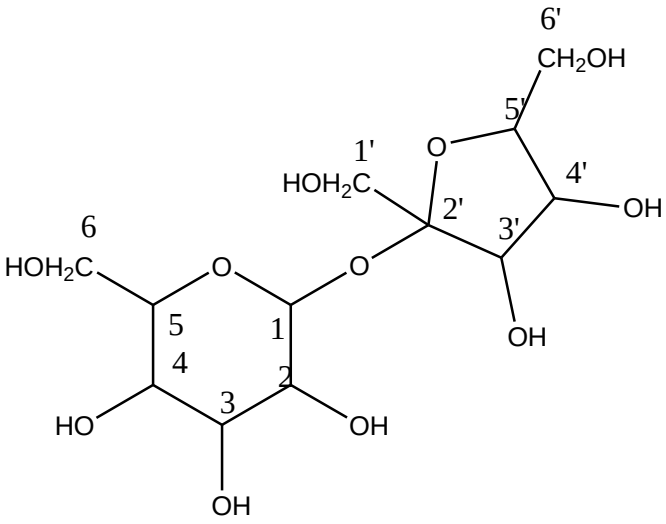


Figure 9: The proposed structure of **LSM-11**

4.6. Result of GC-MS Analyses of n-hexane Extracted Seed Oil of *L. sativum*

The components of the oil were identified using GC-MS analysis after converted in to FAME. The GC-MS spectrum of the seed oil was shown in **Appendix 10** and its components were given as in **Table 7.**

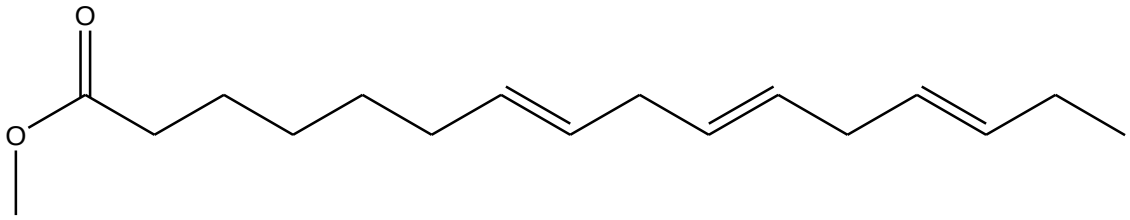
Table 7: GC-MS analysis of n-hexane extracted seed oil of *L. sativum.*

Retention time	Compound name	Molecular formula	Molecular weight	Area %
6.313	Acetamide,2-fluoro-	C_2H_4FNO	77	0.67
12.351	1-methyldodecylamine	$C_{13}H_{29}N$	199	0.56
13.632	Methylpent-4-enylamine	$C_6H_{13}N$	99	1.01
15.934	Benzeneethanamine,4-fluoro-.beta.,3-dihydroxy-N-methyyl-	$C_9H_{12}FNO_2$	185	1.33
16.291	1-propanamine,N,2-dimethyl-	$C_5H_{13}N$	87	1.06
18.900	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	15.14
21.679	Acetamide,N-(aminocarbonyl)-2-chloro-	$C_3H_5ClN_4O_2$	136	4.9
21.796	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	64.42
22.230	Benzeneethanamine,2-fluoro-.beta.,5-dihydrooxy-n-methyl-	$C_9H_{12}FNO_2$	185	3.74
25.695	Amphetamine	$C_9H_{13}N$	135	5.79
26.228	1- Butanamine, N-methyl	C5H13N	87	1.38

The result obtained from the GC-MS analysis indicated that, the n-hexane extracted seed oil of *L.sativum* contained 11 different types of components. The major component was 7,10,13-hexadecatrienoic acid(64.42%) unsaturated fatty acid. The seeds oil were also containing saturated fatty acid (hexadecanoic acid 15.14%), nitrogen containing aromatic derivative compounds and nitrogen containing hydrocarbons.

Based on the GC-MS analysis result, the structure of components were identified from **LSH** extracted seed oil of *L.sativum* given as followed in their abundances order.7,10,13-hexadecatrienoic acid, methyl ester (35, 64.42%), hexadecanoic acid, methyl ester (36, 15.14%), amphetamine (37, 5.79%), acetamide, N-(aminocarbonyl) -2- chloro-(38,4.9%), benzeneethanamine, 2-fluoro-. beta., 5-dihydrooxy-n-methyl (39, 3.74%), 1- butanamine, N-methyl (40, 1.38%), benzeneethanamine, 4-fluoro -. beta., 3-dihydroxy-N-methyyl- (41, 1.33%),

1-propanamine, N, 2-dimethyl- (42, 1.06%), methylpent-4-enylamine (43, 1.01%), acetamide, 2-fluoro- (44, 0.67%), 1-methyldodecylamine (45, 0.56%).



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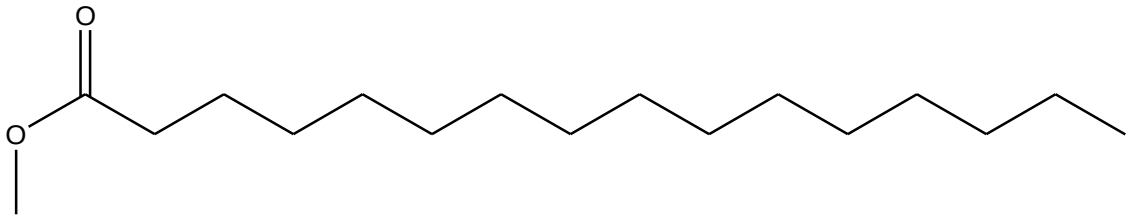

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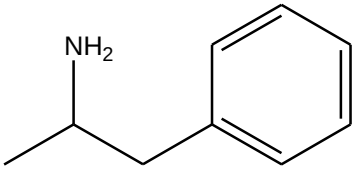

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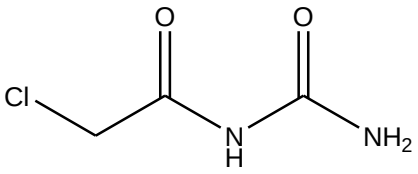

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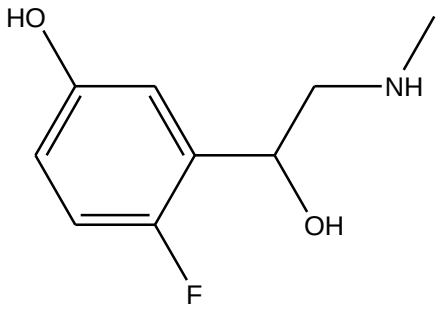

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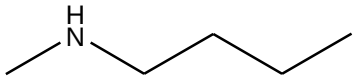

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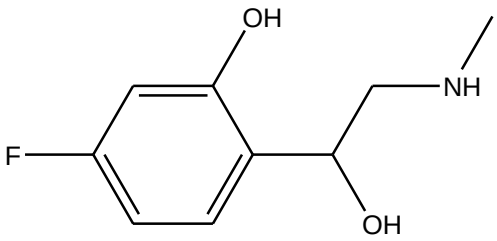

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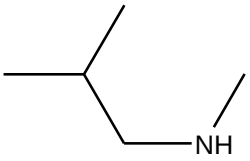

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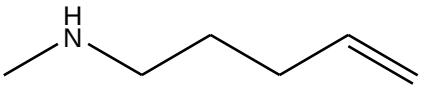

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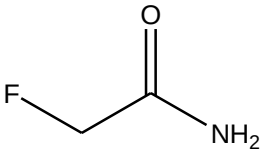

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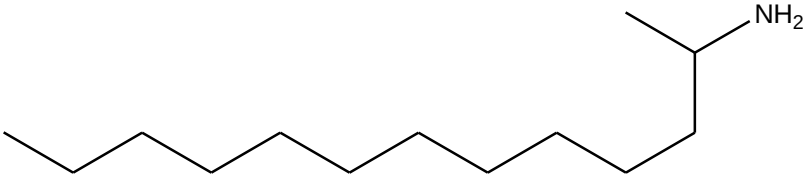

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Figure 10: Structure of components obtained in **LSH** extract seed oil.

4.7. Result of the GC-MS Analysis of the Essential Oil of *L. sativum*.

The components of the essential oil were identified using GC-MS analysis. The GC-MS chromatogram of the seed oil was shown in **Appendix 11** and its components were given as in **Table 8**.

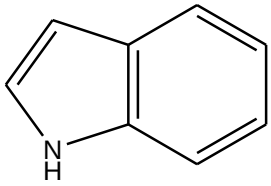
Table 8: The GC-MS analysis of the essential oil of *L. sativum*

Retention time	Compound name	Molecular formula	Molecular weight	Area %
8.702	Indole	C_8H_7N	117	63.78
11.756	Thiocyanic acid, phenylmethyl este	C_8H_7NS	149	5.23
12.032	Benzene, (isothiocyanatomethyl)-	$C_8H_7N_5$	149	6.95
12.352	Tetradecane	$C_{14}H_{30}$	198	0.49
13.631	pentadecane	$C_{15}H_{32}$	212	0.68
15.931	2H-Azepin-2-one,hexahydro-1-methyl-	$C_7H_{13}NO$	127	0.17
16.292	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.07
18.898	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7.36
21.682	1-Methyl-2-phenoxyethylamine	$C_9H_{13}NO$	151	1.42
21.782	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	10.67
22.232	Methyl stearate	$C_{19}H_{38}O_2$	298	2.18

The result revealed, the plant seed contained 11 different types of components. Indol (63.78%), was a major component of the essential oil of *L. sativum* seed. Moreover, the analysis result showed, the seeds oil was also contained saturated and unsaturated fatty acids (21.28%),nitrogen and sulphur containing aromatic derivative compounds(12.18%), saturated hydrocarbons (1.35%), nitrogen and oxygen containing aromatic derivative compound (1.24%) and nitrogen containing ketone (0.17%).

Based on the GC-MS analysis result, the structure of components were identified from *L.sativum* seed essential oil given as followed in their abundances order. indole (46, 63.78%),9-

octadecenoic acid (Z)-, methyl ester (47, 10.67%), hexadecanoic acid, methyl ester (48, 7.36), benzene, (isothiocyanatomethyl)- (49, 6.95%), thiocyanic acid, phenylmethyl ester (50, 5.23%), methyl stearate (51, 2.18%), 1-methyl-2-phenoxyethylamine (52, 1.42%), methyl tetradecanoate (53,1.07%),pentadecane(54,0.68%),tetradecane(55, 0.49%), 2H-azepin-2-one, hexahydromethyl- (56, 0.17%).



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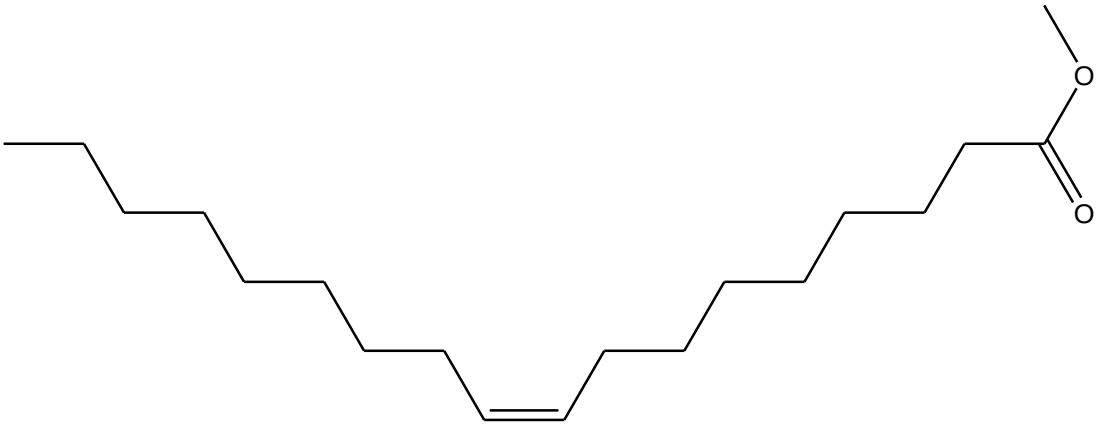

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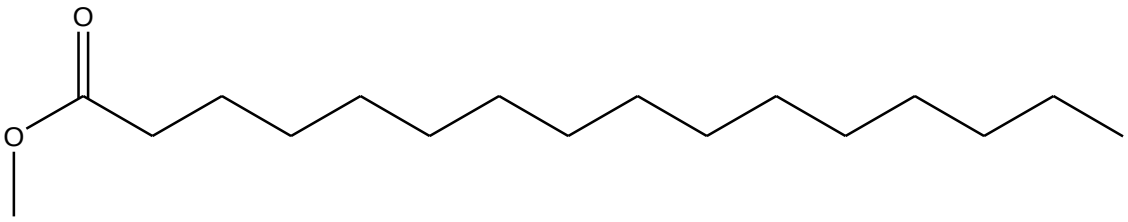

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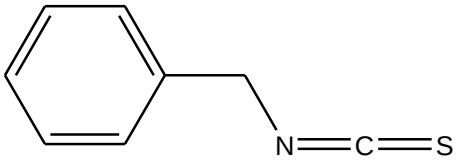

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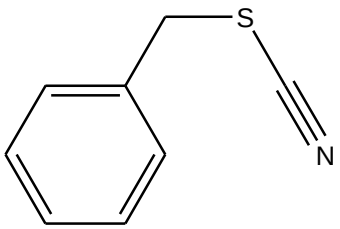

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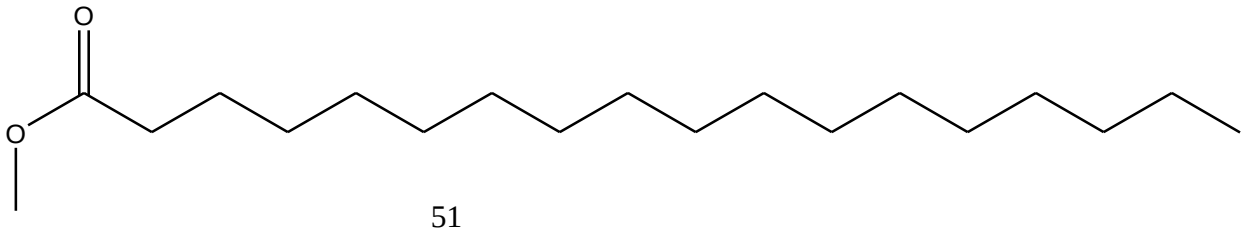

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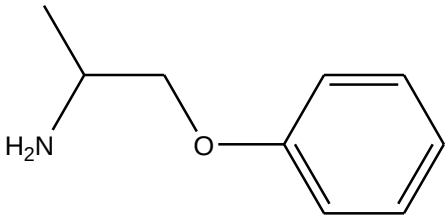

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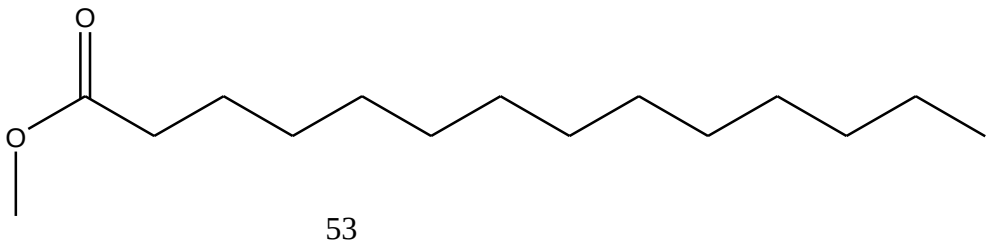

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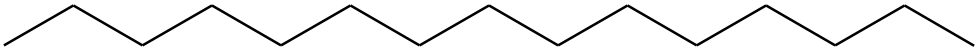

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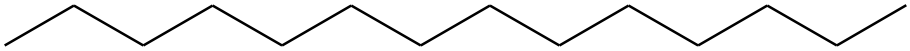

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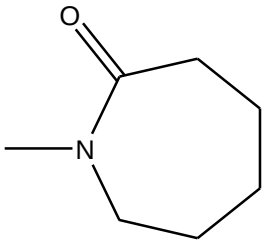

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Figure 11: Structures of components of **LSE** oil

4.8. Result of Antibacterial activity tests

The result revealed the methanol crude extract was not showed inhibition activities against all tested bacteria. **LSM-9** showed moderate antibacterial activities against the tested Gram negative bacteria such as: *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. On the other hand, the essential oil showed great antibacterial activities against gram positive bacterium; *Stphylocous aureus* and in all tested gram negative bacteria recording higher inhibition zone. In addition, the bioassay result indicated ceftriaxone*;* standard medicine was higher inhibition zone than the methanol crude extract and **LSM-9**.Whereas, the essential oil inhibited more than ceftriaxone in *Stphylocous aureus*, *E.coli* and *Proteus mirabilis*.

Table 9: Result of inhibition zone of tested samples against bacteria

No	sample	Concentration (mg/mL)	Bacteria and inhibition zone			
			Stphylococus aureus	*Escherichia coli*	*Proteus mirabilis*	*Klibsiella pneumeniae*
1	LSM-9	1	6 mm	11 mm	10 mm	9 mm
2	Methanol crude ex.	1	6 mm	6 mm	6 mm	6 mm
3	Essential oil	1	60 mm	45 mm	40 mm	18 mm
4	Ceftriaxone	0.03	23 mm	23 mm	23 mm	23 mm

Key: 6 mm = Resistance > 6 mm = Sensitive

0.03 mg/mL concentration of ceftriaxone, standard medicine (positive control) was used and its inhibition zone 23 mm in all of the tested strains. 20 μL of each of the tested sample was taken and added to the 6mm whole made on the disc using a micropipette.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The result of the present study indicated that the methanol crude extract of the seeds of *L. sativum* was subjected to silica gel column chromatography and afforded two compounds labeled as **LSM-9** and **LSM-11**. GC-MS analysis of the hydrodistilled seed oil and the n-hexane extract oil of *L. sativum* seed showed the presence of 11 components in each type of oil. In addition, the result of preliminary phytochemical screening test indicated more phytoconstituent present in the methanol extract than other solvent extracts (n-hexane, dichloromethane, ethyl acetate). Antibacterial activities were performed using disc diffusion method and the result showed that **LSM-9** was active against the tested Gram negative bacteria and the methanol crude extract did not show inhibition activities against the tested Gram positive and Gram negative bacteria. Whereas, the essential oil was active against the tested Gram positive and Gram negative bacteria.

5.2 Recommendations

As revealed from the TLC analysis of the methanol extract, the two isolated compounds are not the only compounds can identified from the extract. Therefore, further study should take place on the methanol extract to drive out new compounds. In addition, as the study result confirmed, the antibacterial activity of the essential oil was very strong. Therefore, experimental test need to be conducted on the antifungals and antioxidant activity of the essential oil for the future.

6. Reference

1. Sarker, S.D., Latif, Z. and Gray, A.I. (**2006**). 'Natural Product Isolation', in Natural Product Isolation 2nd ed, Human Press, Totowa, New Jersey. pp. 1-25.
2. Newman, D.J. and Cragg, G.M. (**2012**). Natural products as sources of new drugs. Journal of Natural Product, 75: 311–335.
3. WHO (**2002**). World Health Organization traditional medicine strategy, 2002-2005. Geneva: WHO publication.
4. Gurib-Fakim, A. (**2006**). Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. Molecular aspects of Medicine, 27, 1-93.
5. Ly, T.N., Yamauchi, R., Shimoyamada, M. and Kato, K. (**2002**). Isolation and Structure elucidation of some glycosides from the rhizomes of smaller galangal (Alpina). Journal of Agricultural and Food Chemistry.p.179-181.
6. Sofowora, A. (**1982**). Medicinal plants and traditional medicine in Africa. John Willey and sons Ltd., New York, 3-26.
7. Agarwala, R.N. (**2015**). Phytochemical Analysis of some medicinal plants. Journal of phytology, 3(12); 10-14.
8. Hamilton, A. (**2003**). Medicinal Plants and Conservation: Issues and approaches. Panda House, Catteshall Lane, London. International Journal of Remote Sensing, 21(12), 2389-2402.
9. Leonti, M., Vubrans, H., Sticher, O. and Heinrich, H. (**2001**). Ethnopharmacology of the pepoluca, Mexico an evaluation. Journal of pharmacy and pharmacology 53 (12); 1653-1669.
10. Abiyot, B., Zemede, A. and Ensermu, K. (**2006**). Ethnobotany of plants used as insecticides, repellents and anti-malarial agents in Jabitehnan district, West Gojjam. Ethiop. J. Sci., 29(1):87–92.
11. Solomon, G. (**2014**). Phytochemical screening and antimicrobial activities of crude extract of *L. sativium* seeds grown in Ethiopia. Inter. J of pharmac. Sci. and Res., 5(10): 4182-4187.
12. Said, M.H. (**2012**). Genetic Diversity Study of *L. sativum* Populations from Ethiopia Using Morphological Characters and ISSR Markers, AAU, Addis Ababa, Ethiopia.

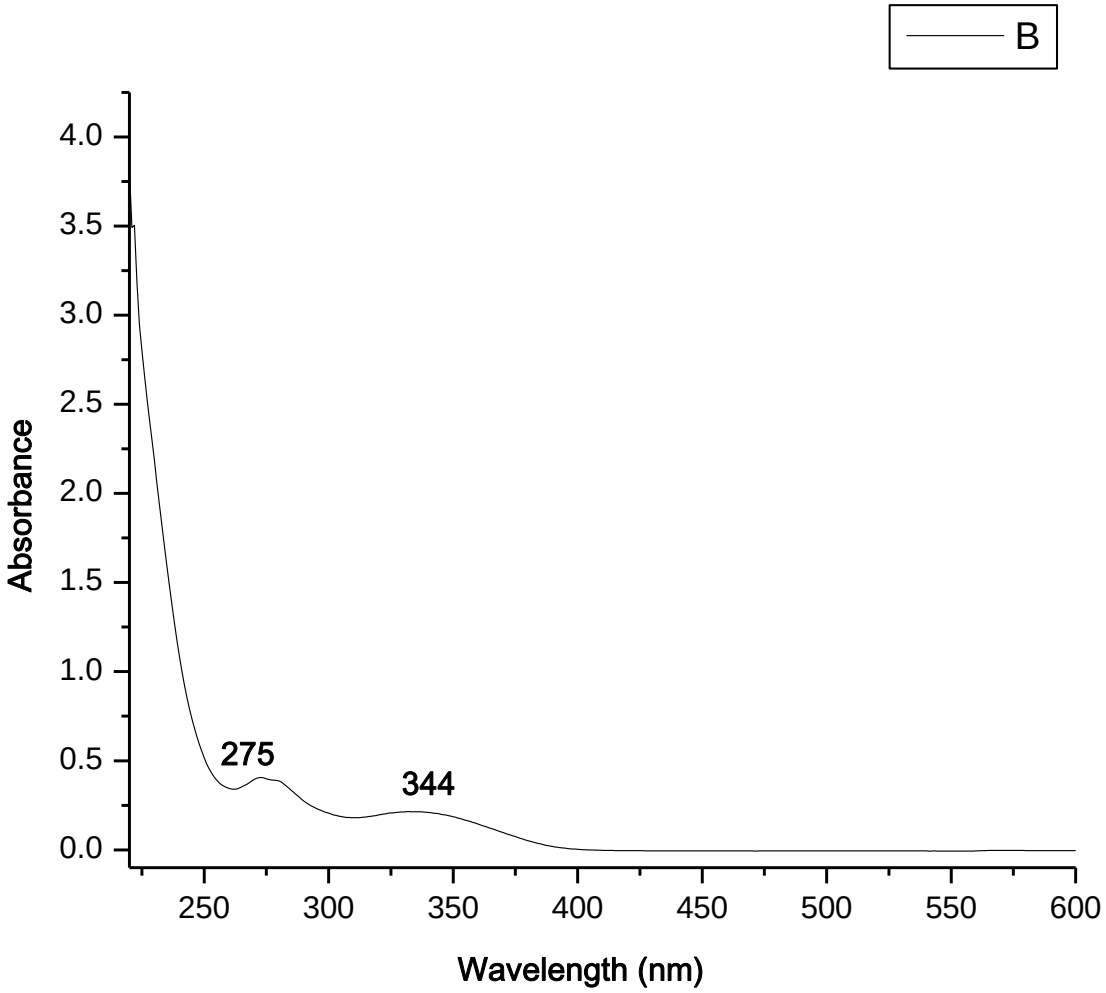
13. Cragg, G.M. (**1997**). Screening of natural products of plant, microbial, and marine origin: The NCI experience. In phytochemical Diversity: A source of New Industrial products; 137-138.
14. Vlietinck, A.J., Apers, S. (**2001**) Biological screening methods in the search for pharmacologically active natural products. In Bioactive compounds from Natural sources: Isolation, characterization, and biological properties; 104-106.
15. Peterka, S.U. and E. Schlosser, (**1986**). Biol Bunde Sans taltf. L and und forst wirtscheft, 232, 278.
16. Mithen, R.F. and Lewis, B.G. (**1986**). In vitro activity of glucosinolates and their products against *Leptosphaeria maculans*. Trans. Br. Mycol. Soc. 87, 433–440.
17. Vang, O. (**1994**). Neue Aspecte Gesund wirkung pflanz. Nahrungsn vortragstage. Dtsch. Ges. Qualitataetes forsch. 29th, 74-85.
18. Kirtikau, K.R. and Basu, L. (**1984**). Indian Medicinal plants, vol.1—V, 2nd ed., Bishen Singh Mahendra pal singh, Dehra Dun, India, 123-132
19. Malik, R.S., I.J. Anand, Srinvasachar, S. (1983). Effect of glucosinolates in relation to aphid (lipaphis erysimi Kalt.) fecundity in Crucifers. Ind. J. Trop. Agric., 1: 273-278.
20. Loatfy, B., (**1999**). "Flora of Egypt" Volume I, Al Hadara publishing, Cairo, Egypt, 7.
21. Tackholm, V., (**1974**). "Student Flora of Egypt". Published by Cairo University printed by cooperative printing company, 8.
22. Faulkner, H. W. (**1917**). The Mysteries of the Flowers. Frederick A. Stokes Company. p. 238. Page 210
23. Mummenhoff, K. (**2001**). Chloroplast DNA phylogeny and biogeography of *L.* (Brassicaceae). American Journal of Botany 88(11), 2051-63.
24. Mukherjee, (**2002**). Quality control of herbal drugs. Business Horizons pharmaceutical publishers New Delhi. ISBN 81-900788-4-4
25. U-H maier, H. Gundlach, M.H. zenk, (**1998**). Seven imidazole alkaloids from *L. sativum.* Phytochemistry, 49, 1791-1795.
26. Muhammad, I.; Zhao, J.; Khan, I.A. (**2010**). Maca (*L. meyenii*). *Research gate*, 59(1), 435–443.

27. Aranda, E., Garcıa-Romera, I., Ocampo, J.A., Carbone, V., Mari, A., Malorni, A., Sannino, F., De Martino, A., Capasso, R. (**2007**). Chemical characterization and effects on *L. sativum* of the native and bioremediated components of dry olive mill residue. Chemosphere, 69, 229-239
28 Shrinivas K, prakesh K, kiran HR, Prasad PM, (**2003**). Study of ocimumbasilicum and plantago ovate as disintegrants in the formulation of dispersible tablets. Indian J pharm. Sci.; 65, 180-3.
29 Qudihapankaj, (**1998**). Medicinal plant – chandrasoor, national scientist Academy of India. Agricultural Department, pp. 44.
30 Hiba, F., Wasfieh, N.N. and Haneen, N. (**2014**). A Review Article L. sativum (garden cress).Research Gate, 1-3.
31 Sheel, S., and Nidhi A. september (**2011**) Nourishing and healing powers of garden cress (*L. sativum* Linn) A review, Indian journal of natural products and resources, pp, 292-297.
32 Divanji, M., Viswanatha, G.L., Nagesh, S., Vishal, J., and Shivaprasad, H.N. (**2012**). Ethnopharmacology of *L. Sativum* Linn (Brassicaceae): A Review. International Journal of Phytothearpy Research. 2(1): 1-7.
33 Kloos. H., (**1976**) "Preliminary studies of medicinal plants and plant products in Ethiopian markets," Journal of Ethiopian Pharmaceutical Association,vol.2,pp.18-28.
34 Nadkarni. K.M., (**1976**) Indian Materia Medica, Popular Prakashan, Bombay, India
35 Duke. J. A., Bogenschutz-Godwin. M. J., Ducelliar. J., and Duke. P.A.K., (2002) Handbook of Medicinal Herbs, CRC Press, Boca Raton,Fla, USA.
36 Jean. S. J., Ferdinand. C., (2003) The Medicinal and Poisonous plants, India, Scientific Publishers, Jodhpur, India. Pp.260.
37. Nandkarni KM., (**1954**). The Indian medica. 2nd ed. Mumbai; dhootapa-peshwarprakashan
38. Chopra, R.N., Nayar, S.L. & Chopra, I.C. (1956). "Glossary of India Medicinal Plants", Council of Scientific and Industrial Research, New Delhi, 46-47.
39. Jyoti Agarwal & D.L.Verma (**2011**). Antioxidative Avtivity and Flavonoid Composition from *L. Sativum*. Nature and Science; 9(7): 21 – 25.

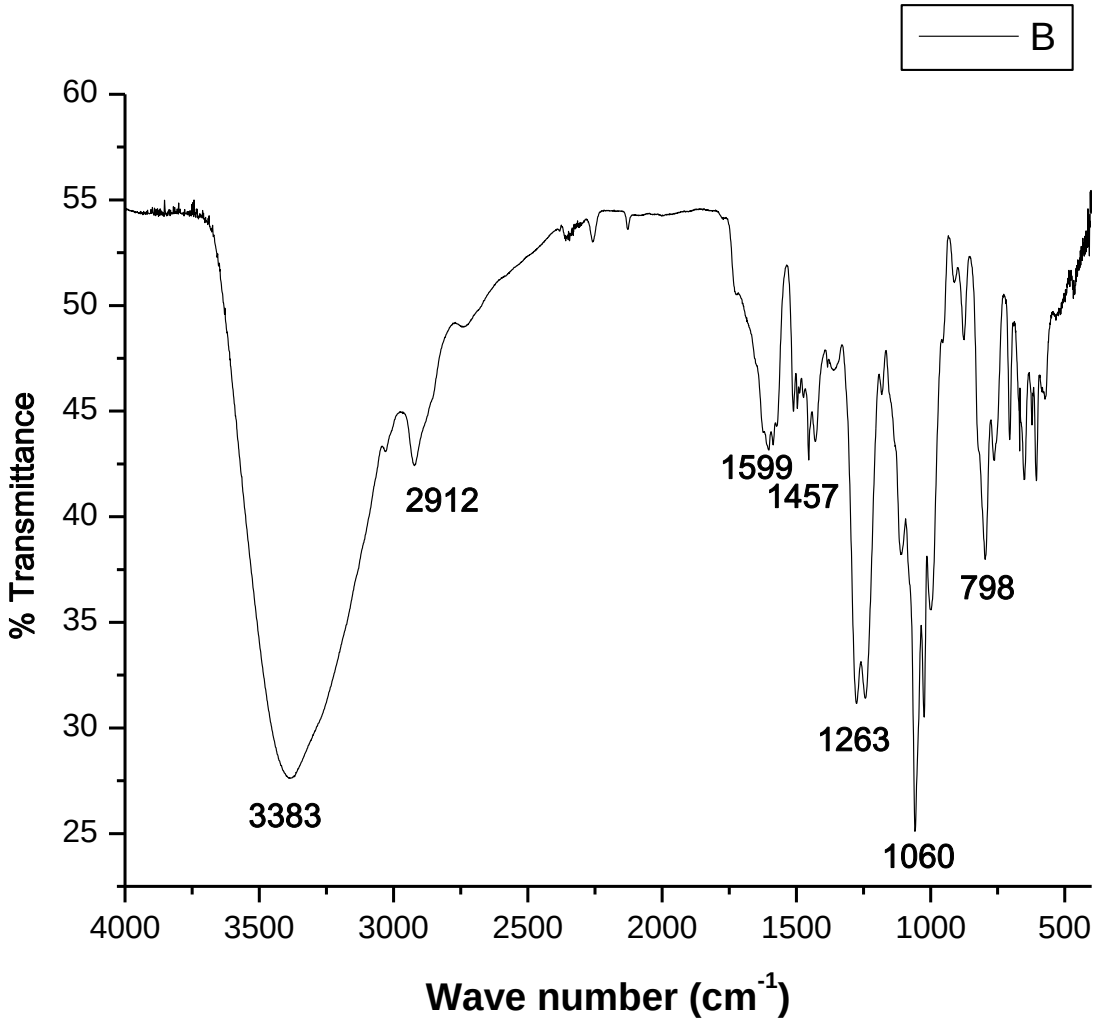
40. Nath D, Sethi N, Singh RK, Jain AK. (**1992**). Commonly used Indian abortifacient plant with special reference to their teratologic effects in rats. J. Ethnopharmacol. 36:147 – 54.
41. Chenna Kesava Rao, A.T., Priyanka, B., Maheswari, K., Rambabu, G., and J. Vidya Sagar (**2016**). "Protective effect of ultra-sonic bath assisted methanol extract of *L. sativum* seeds by using chemotherapy induced neuropathy in rats." World Journal of Pharmaceutical Research, 5(12), 205-233.
42. ISO12966-2 (**2011**): Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters-part 2: preparation methyl esters of fatty acids.
43. Evans', W. (**1996**). Trease and Evans Pharmacognosy, 14th edition, WB Saunders company Ltd; London. Pp. 224–228, 29–309, 542–575.
44. Harborne, J. (**1998**). Phytochemical methods. A guide to modern techniques of plant analysis, 3rd Edition, Chapman and Hall In New York, 1-15.
45. Nostro, A., Germanŏ, M.P., D'Angelo, V., Marino, A.and Cannattelli, M.A. (**2000**): Extraction Methods and Bioautography for Evaluation of Medicinal plants for Antimicrobial activity. Lett. Appl. Microbiol., 30:379-384.
46. Bones AM, Rossiter JT (2006). The enzymic and chemically induced decomposition of glucosinolates. Phy 67(11):10 doi:10.1016/j.phytochem.2006.02.024.
47. Martı´nez-Ballesta MC, Moreno DA, Carvajal M (2013). The physiological importance of glucosinolate on plant response to abiotic stress in Brassica. Int J Mol Sci 14(6):1160711625 doi:10.3390/ijms140611607.
48. Mithen RF, Dekker M,Verkerk R, Rabot S, JohnsonIT(2000) The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J Sci Food Agr 80(7):967–984.

7. Appendices

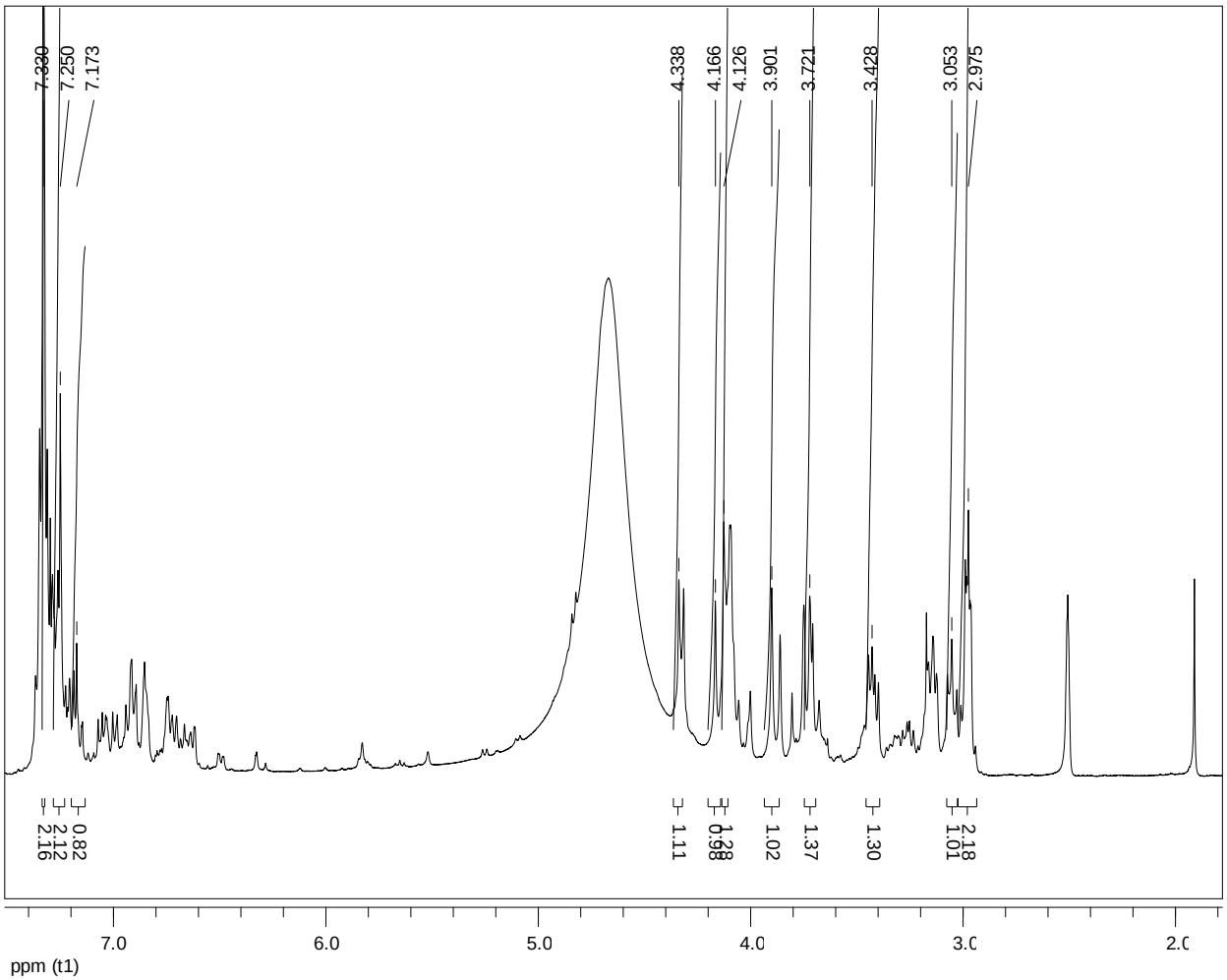
Appendix 1: UV-Vis spectra of **LSM-9**



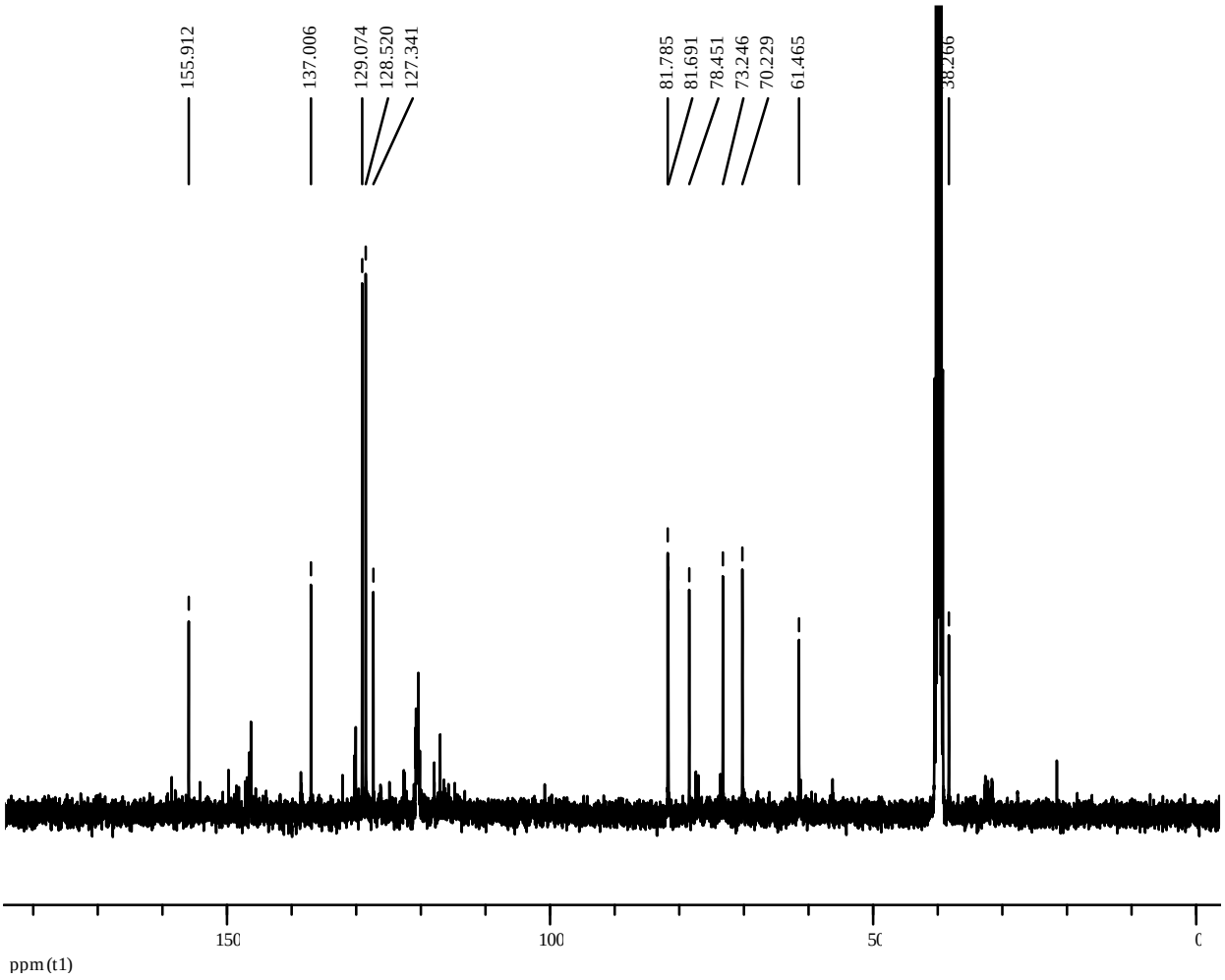
Appendix 2: IR spectrum of compound **LSM 9**



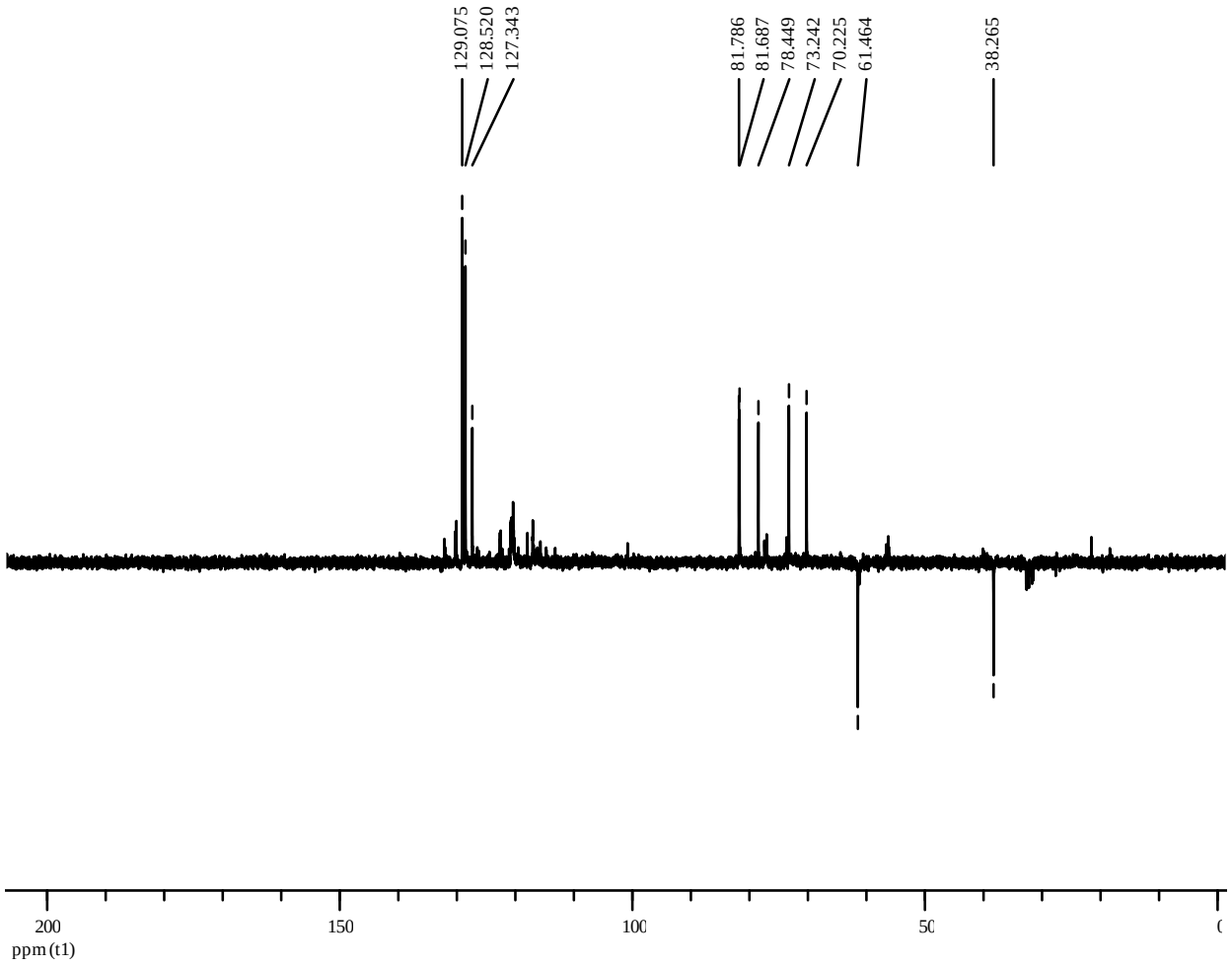
Appendix 3: ^{1}H- NMR spectra of **LSM-9**



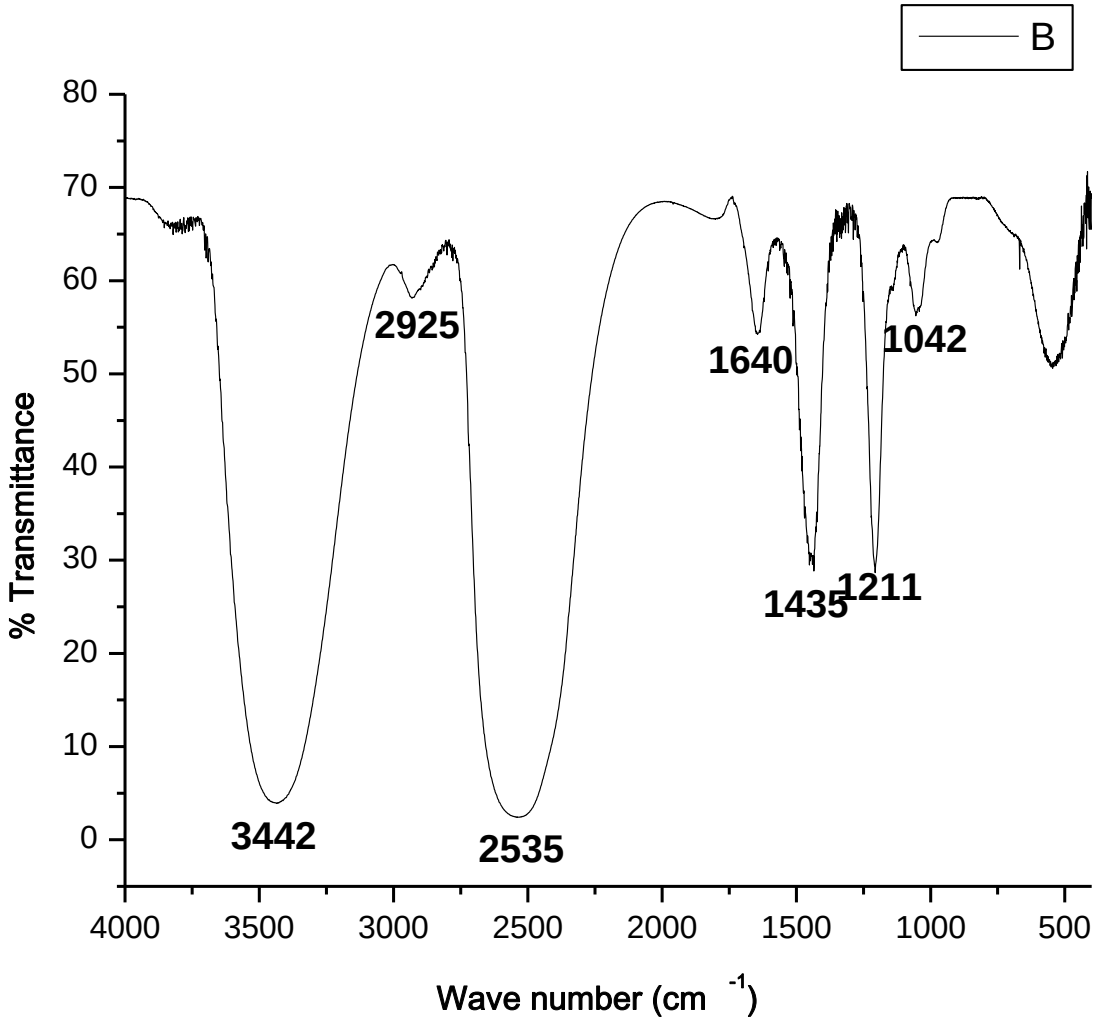
Appendix 4: ^{13}C- NMR spectra of **LSM-9**



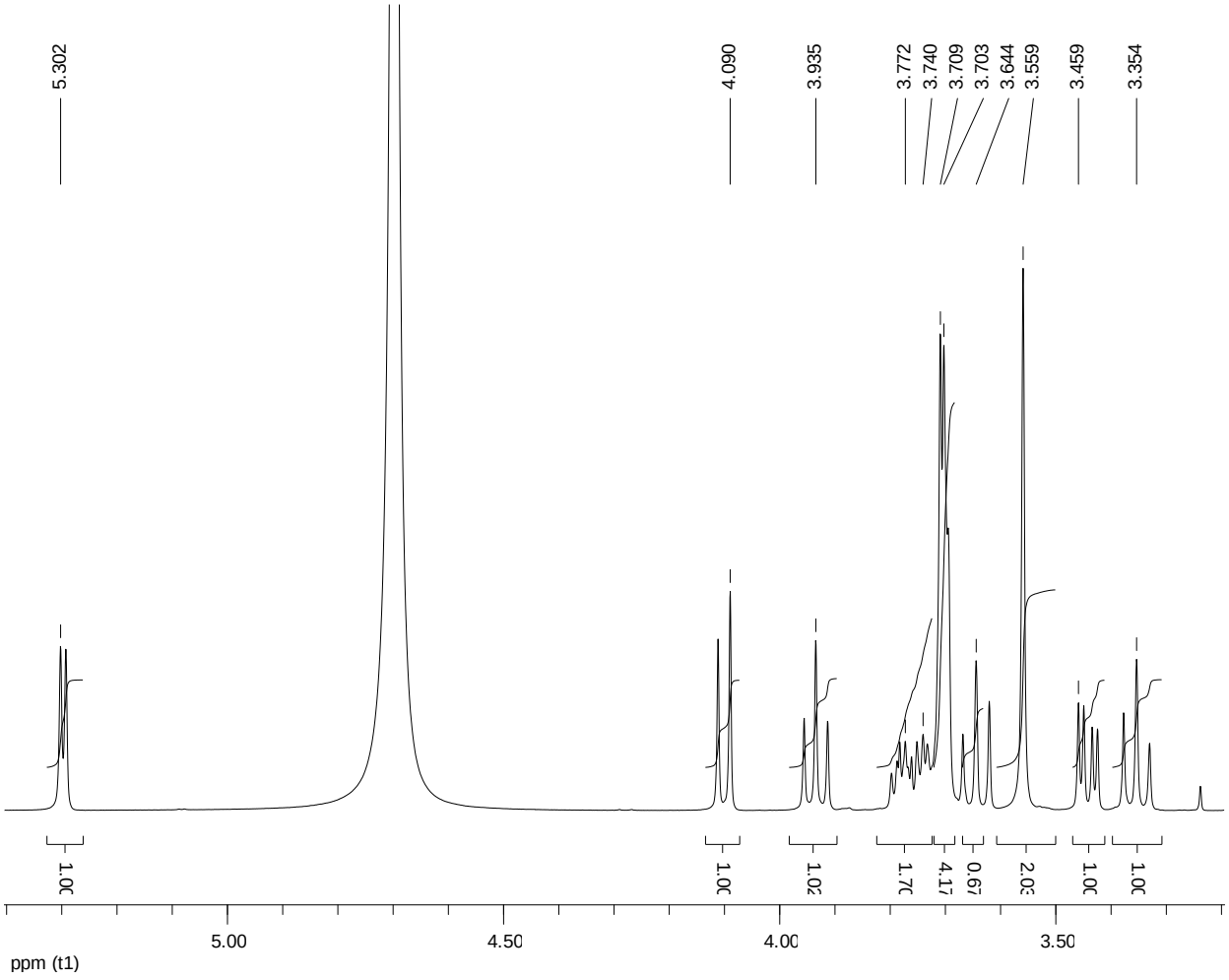
Appendix 5: DEPT-135 spectra of **LSM-9**



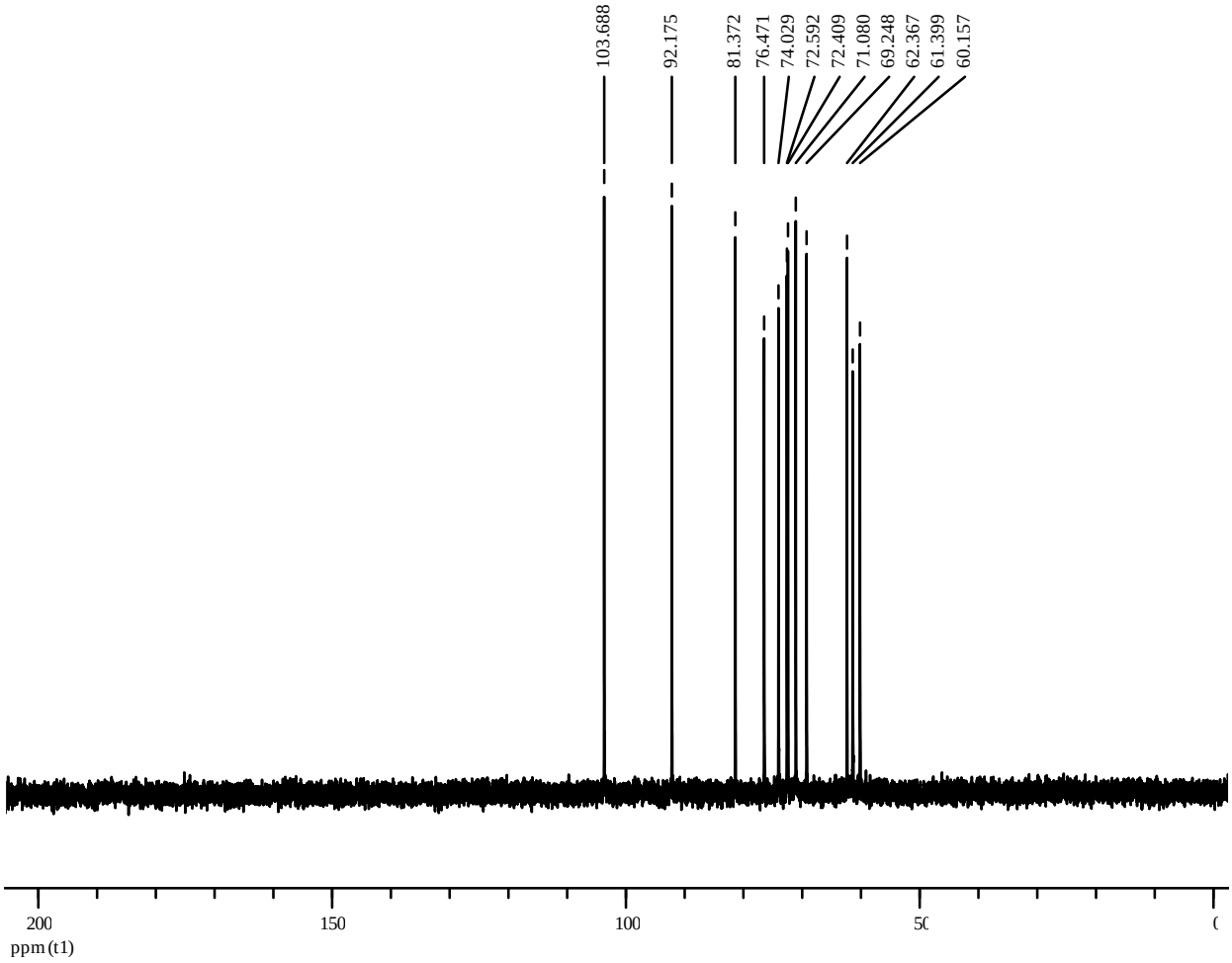
Appendix 6: IR spectra of **LSM-11**



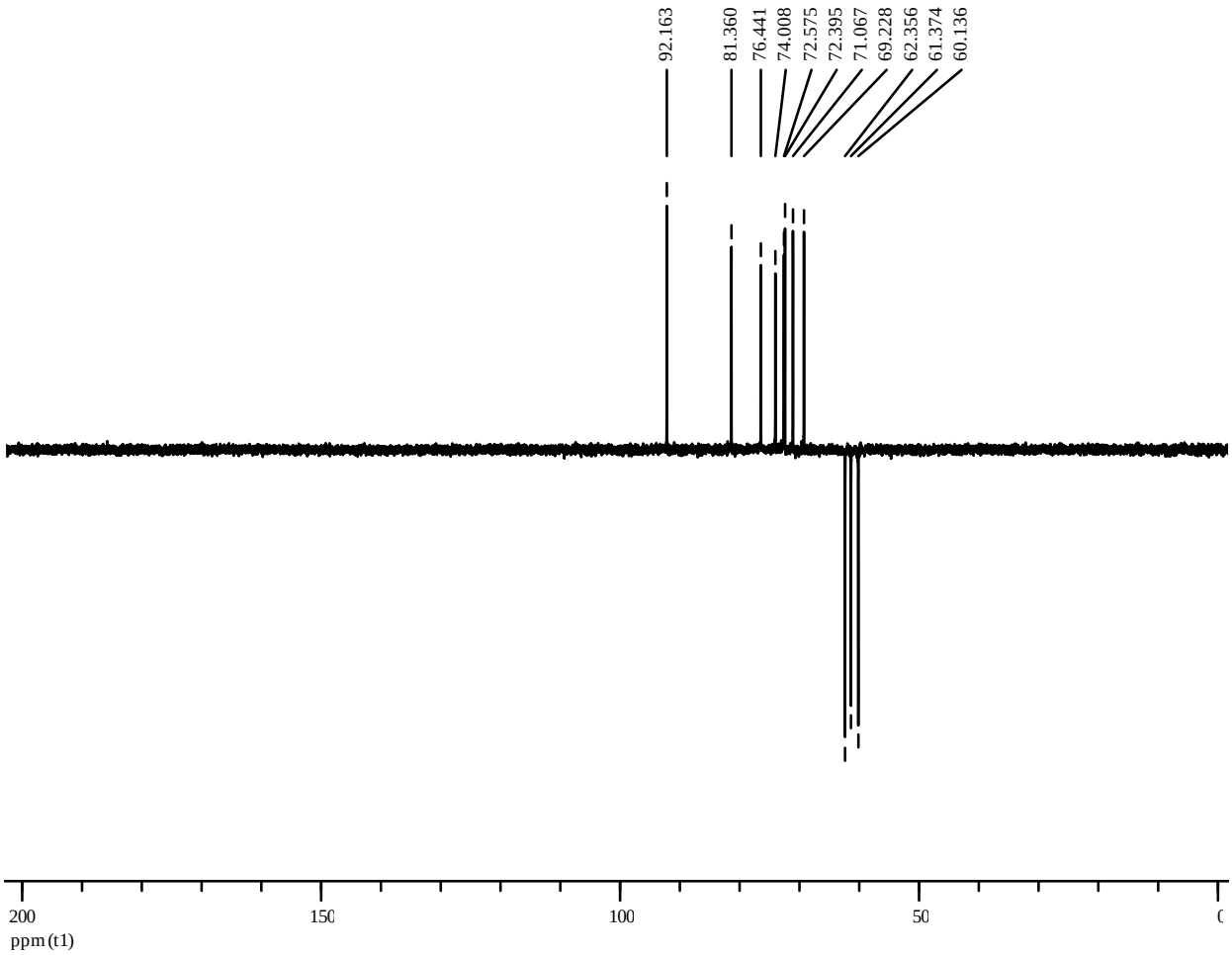
Appendix 7: ^{1}H- NMR spectra of **LSM-11**



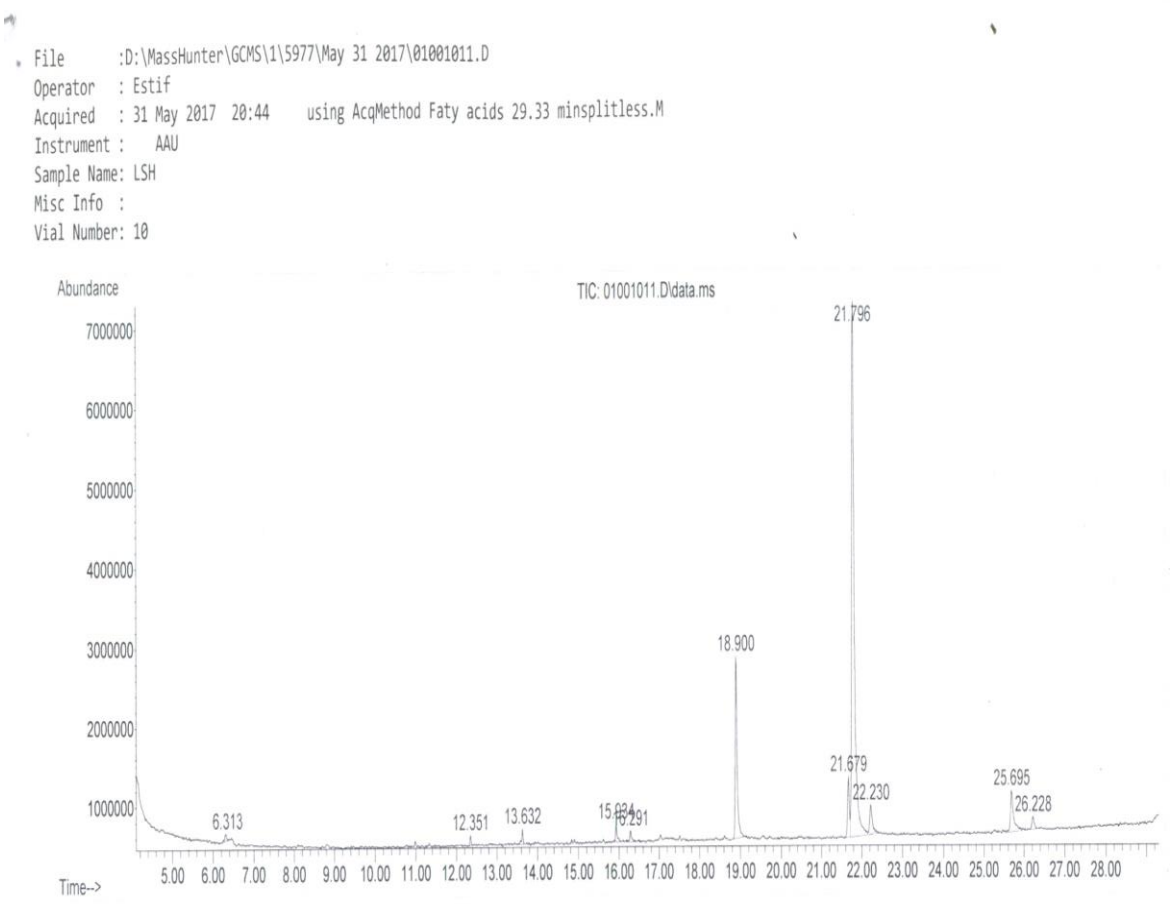
Appendix 8: ^{13}C-NMR spectra of **LSM-11**



Appendix 9: DEPT-135 spectra of **LSM-11**



Appendix 10: GC-MS spectral data of n-hexane extract seed oil of *L. sativum.*



Appendix 11: GC-MS spectral data of *L. sativum* essential oil