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ISOLATION AND CHARACTERIZATION OF BIOACTIVE MOLECULES FROM *Lantana camara* FRUITS

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ISOLATION AND CHARACTERIZATION OF BIOACTIVE MOLECULES FROM *Lantana camara* FRUITS

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

By

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Abbreviations

MHB	Mueller Hinton Broth
DMSO	Dimethyl Sulfoxide
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance Spectroscopy
^{1}H-NMR	Proton nuclear Magnetic Resonance Spectroscopy
^{13}C-NMR	C-13 Nuclear Magnetic Resonance Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
CC	Column Chromatography
TLC	Thin Layer Chromatography
CFU	Colony Forming Unit

Abstract

The aim of this research study was to carry out isolation and characterization of bioactive compounds from *Lantana camara* (Verbenaceae) fruits. The plant material was collected from the Jimma University Institutes of Technology. The dried and powdered plant material (fruits) was subjected to sequential solvent extractions (petroleum ether, chloroform, acetone and methanol) using maceration techniques. Antibacterial evaluation of crude extracts was carried out using *in vitro* method against four standard bacterial species (viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimurium*). Among the four crude extracts, the chloroform extract showed the highest antibacterial activity and was subjected to column chromatographic separation that led to isolation of five compounds (labeled as **LC-1**, **LC-2**, **LC-3**, **LC-4** and **LC-5**). Of which, three compounds named *β*-sitosterol (**LC-1**), di-2-ethylhexyl-phthalate (**LC-4**) and trilinolein (**LC-5**)) were fully characterized based on the observed spectroscopic data (^{1}H-NMR, ^{13}C-NMR, DEPT-135 and IR) data and comparison with reported data in literature. The compounds were also evaluated for their antibacterial activities and exhibited activity superior to standard antibiotic (gentamicine). Thus, the observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections.

Key words: *Lantana camara* fruits; Extraction; Isolation; Antibacterial activity; β-Sitosterol; Di-2-ethylhexyl-phthalate; Trilinolein.

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1. INTRODUCTION

1.1. Natural products

Natural product could be defined as a chemical compound that produced naturally by plants, animals and microorganisms. These chemical products of living organisms can be classified into primary and secondary metabolites. Primary metabolism refers to the processes producing the carboxylic acids of the Krebs cycle, an amino acids, carbohydrates, fats, proteins and nucleic acids, which are present in all plants and essential for the survival and well-being of the organism[1]. Secondary metabolites, on the other hand, are non-essential to life but contribute to the species' fitness for survival[1]. It also produced using other metabolic pathways than primary metabolites. These pathways are more characteristic for the particular family or genus and are related to the mechanism for evolution of species. In fact, the specific constituents in a certain species have been used to help with systematic determination, groups of secondary metabolites being used as markers for botanical classification[1]. Some of these secondary metabolites serve as defensive compounds against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or reducing the growth of nearby competing plants[2].

Secondary metabolites have always been a significant source of new lead compounds in pharmaceutical industries. It has a long tradition as drugs to treat infectious diseases. They still play an important role in modern drug discovery since they represent an immensely rich arsenal of complex structures and a valuable source of new bioactive compounds. About half of the drugs currently in clinical use are natural products (also called secondary metabolites) or synthetic molecules based on natural product scaffolds. It continues to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug is designed[3]. Nature has been a source of medical agents for thousand of year and an impressive number of modern drugs which have been isolated from natural sources; many of this isolation were based on the uses of the agents in traditional medicine. Natural products isolated from plants have been providing noble and clinically active drugs[2]. This plant-based, traditional medicine system continue to play an essential role in health care, with about 80% of the worlds inhabitants relying mainly on traditional medicines for their primary health care[4]. According to

world health organization (WHO) medicinal plants could be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand of their properties, safety and efficacy[5]. Approximately 20% of the plants in the world have been submitted to pharmacological or biological test and a substantial number of antibiotics introduced on the market are obtained from natural or semi synthetic resources[6]. Over 50% of all modern clinical drugs are of natural origin and natural products play an important role in drug development in the pharmaceutical industry[7]. Plants consist of a number of biologically active ingredients such as alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds[8]. These phytochemicals are synthesized in all parts of the plant body and are mainly attributed to the pharmacological actions. Medicinal plants are usually screened for phytochemicals that may lead to its further isolation, purification and characterization of active principle. The active compound can be then used as the basis for a new pharmaceutical product[9].

In Ethiopia, medicinal plants play major supplementary roles to the limited modern health care available. The rich traditional knowledge of the people has over the centuries led to the application of plants for food, medicine and other uses. In the country, traditional health care is culturally deep rooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective medicinal values for ailments of human and domestic animals. Thus, medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country. The major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions and that the trust the communities have in the medicinal values of traditional medicine and relatively low cost in using them. Medicinal plants obtained from wild habitats are found in different natural ecosystems of the forests, grasslands, woodlands, wetlands, in field margins and garden fences, as weeds and in many other microhabitats from where they are harvested when the need arises[10].

Lantana camara is one of the plants grows in Ethiopia and an ornamental plant used in traditional medicine for the treatment of various diseases and it is known for the presence of many biologically active compounds. Over the last twenty-five years, a large number of plant species have been evaluated around the world for their antibacterial activities[11]. One of the plants known for having many medicinal uses in traditional system of medicine is *Lantana camara*[11]. All parts of this plant have been used traditionally for several ailments throughout the world. For

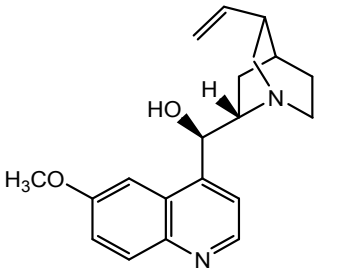
instance, the leaves of this plant were used as an antitumeral, antibacterial, and antihypertensive agent, roots for the treatment of malaria, rheumatism, and skin rashes, the fruits are useful in fistula, pustules, tumors, tuberculosis and rheumatism[12, 13]. A tea prepared from the leaves and flowers was taken against fever, influenza and stomach-ache. In Central and South America, the leaves were made into a poultice to treat sores, chicken pox, high blood pressure and measles[14]. Fresh unripe fruits of *Lantana camara* possesses the antioxidant substance which may be potential responsible for the treatment of cancer and tumors[15].

In Ethiopia, *Lantana camara,* its vernacular name is 'Yewof-qolo' (Amharic) and 'Midhan dubara' (Afan Oromo) found all over Ethiopia mostly on fertile sandy and light clay soil. It has been propagated by running water and birds, which feed on it and thereby it become a major weed in agricultural areas. Fruits are edible; the fresh and ripe is commonly eaten by children in dry area. In different region of Ethiopia, *Lantana camara* is well known as most traditional medicine. For example, northern region, south Gonder and southern region Wonago district it is the most important traditional medicine in treating diarrhea[16, 17]. Literature report showed that *Lantana camara* is one of the exotic species that is invasive in dry lands of Ethiopia. However, people are using it for food, medicine and other multiple purposes. Some people also showed their negative perception for its edibility. When eaten in excess amounts it is also perceived as poisonous while edible by children and currently by adults[18]. Therefore, further study on chemical composition including toxicity of the edible fruit of *Lantana camara* for future use and management is important.

1.2. Natural products in drug discovery

Currently, there is an increasing interest in the study of natural products, especially as part of drug discovery programs, as it represents a formidable reservoir of potentially useful leads for new medicines. The natural products of interest here is organic molecules which are also frequently called secondary metabolites which are produced by various living organisms[3]. Medicinal plants are very high priced for human to exploiting biological activities, cost effectiveness and lesser side effect[19]. Secondary metabolites are rich sources in medicinal plants with exciting biological activities. The use of data on traditional medicine can provide a very valuable short cut by indicating plants with specific folk medicinal uses, which might be likely sources of biologically active compounds [20]. Over the lifetime of the modern pharmaceutical

industry, natural products have been established as excellent sources for the discovery of novel compounds with therapeutic potential [3]. Drugs derived from natural sources have been the corner stone for treating people infected with different disorder. For many years, medicine and natural products have been closely linked through the use of traditional medicines[3]. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as quinine (**1**) isolated from the bark of *Cinchona succirubra*, were the natural product to show efficacy against malaria[21]. The most famous and well known anti-inflammatory agent, acetylsalicyclic acid (**2**) (aspirin) derived from the natural product; salicin (**3**) isolated from the bark of the willow tree *Salix alba* [22, 23].Tubocaurarine (**4**) isolated from the climbing plant, *Chondrodendron tomentosum* (Menispermaceae) is one of the active constituents used as a muscle relaxant in surgical operations, reducing the need for deep anesthesia (Figure 1)[24].



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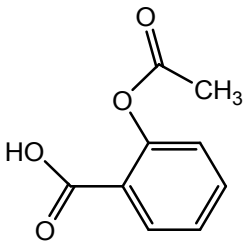

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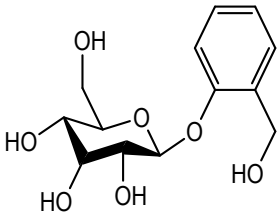

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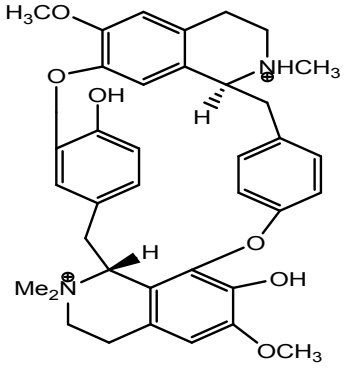

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Figure 1. Drugs derived from medicinal plants[21-24]

1.3. Statement of the problem

The plant *Lantana camara* is well known for the treatment of various ailments and other domestic applications. In Ethiopia, there are a lot of medicinal plants that have potential natural products, but not investigated thoroughly. *Lantana camara* is one of the economically important plants that grow in Ethiopia, and different parts of this plant have been used as a traditional medicine and in some parts of country (East Shewa and Afar) people. Literature survey showed that, among seven species of genus *Lantana* found in the world, one species particularly found in Ethiopia[25]. However, previous studies revealed that *Lantana camara* as a major weed in agricultural land and it is one of the exotic invasive species in dry lands. Thorough searches of literature show that taxonomically the species of *Lantana camara* are notable and hybridization is widespread. Around Jimma, this plant is widely distributed and people use it for different purpose. However, no much work has been reported on the isolation and characterization of bioactive molecule from its fruits. Therefore, the present study was aimed for the evaluation of antibacterial activity, isolation and characterization of bioactive molecules from the fruits of *Lantana camara.*

1.4. Objectives of the study

1.4.1. General objective

- The main objective of this study was to isolate, characterize and evaluate antibacterial activity of compounds from fruits of *Lantana camara.*

1.4.2. Specific objectives

- To isolate the bioactive molecules present in the *Lantana camara* using chromatographic techniques (TLC and column chromatography).
- To assess the antibacterial activities of the crude extracts and isolated compounds from fruits of *Lantana camara* on four pathogenic bacteria strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimurium*).
- To characterize the isolated bioactive compounds, using spectroscopic techniques such as IR and NMR.

1.5. Significance of the study

The bioactive phytochemical constituents present in the plant play a significant role in the development of medicines and drug discovery. Therefore, phytochemical investigation is very important for isolation and characterization of new bioactive molecules from the medicinal plant. Thus, the current study focused on the isolation and characterization of bioactive molecules from *Lantana camara* fruits. The outcome of this study is expected to have the following significances.

- To identify and isolate some compounds that could be used as leads in the discovery of antibacterial agents.
- To give information about the constituents of the plant fruits.
- To document the obtained results from the plant material for further study.

2. Review of literature

2.1.Botanical description *of Lantana camara*

Lantana camara is a heavily branched and evergreen shrub that can grow in compact clumps, dense thickets or as a climbing vine. Stems are square in profile, with small prickles, hairy when young, cylindrical and up to 15 cm thick as it grow older. The leaves are arranged in opposite pairs, they are broadly oval in shape, rough with short hairs, with finely toothed edges. Flowers are a mixture of cream, pink or orange numerous small rounded heads, often in two colours, yellow and red. Fruits are fleshly berries in cluster, green ripening to black (Figure 2)[26].

Figure 2. Picture of *Lantana camara* from its natural habitats

Lantana camara is one of the species that belong to the family Verbenaceae. The family Verbenaceae comprises one hundred genus and about 2600 species distributed in tropical and subtropical regions around the world[25]. Many genera belonging to this family appear to possess

various biological and pharmacological properties. Among the genus, *Lantana camara* is commonly known and widespread species and considered both as a notorious weed and a popular ornamental garden plant. However, it is listed as one of the important medicinal plants of the world[27]. The genus *Lantana* was described by Linnaeus in 1753 and contained seven species, six from South America and one from Ethiopia[25]. Taxonomically, this genus shows difficult classification, because the species are not stable, hybridization is very widespread, the shape of the inflorescence changes with age, and color of the flowers varies with age and maturity[26]. Due to this characteristic, species of this *Lantana* have been much studied with respect to cytogenetic within the family Verbenaceae.

2.2. Medicinal importance of *Lantana camara*

Lantana camara has been used in many parts of the world to treat a wide variety of disorders. Traditionally the plant is used for treating fever, influenza, stomach ache (leaves and flower) , cold, rheumatism, asthma and high blood pressure (whole plant)[27], sores, chicken pox, measles (leaves)[28], stomach ache (powdered root in milk), cough (decoction of leaves), tetanus, rheumatism, malaria and ataxia of abdominal viscera (whole plant), antiseptic for wounds (leaf oil)[26]. Further literature reveals that *Lantana camara* has been reported for fistula, pustules, tumors and rheumatism(fruits)[15],antilymphocytic, immunosuppressive, hepatoprotective, thrombin inhibitory, termiticidal, antimotility, antifilarial, *in-vitro* cytotoxic and antimicrobial activity (different parts of the plant)[29]. The leaf extract has been said to possess the wound healing and antidiabetic property[30]. The pharmacological significance was noted due to the presence of various bioactive compounds such as lantadenes in all *Lantana Camara*. In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities [31].

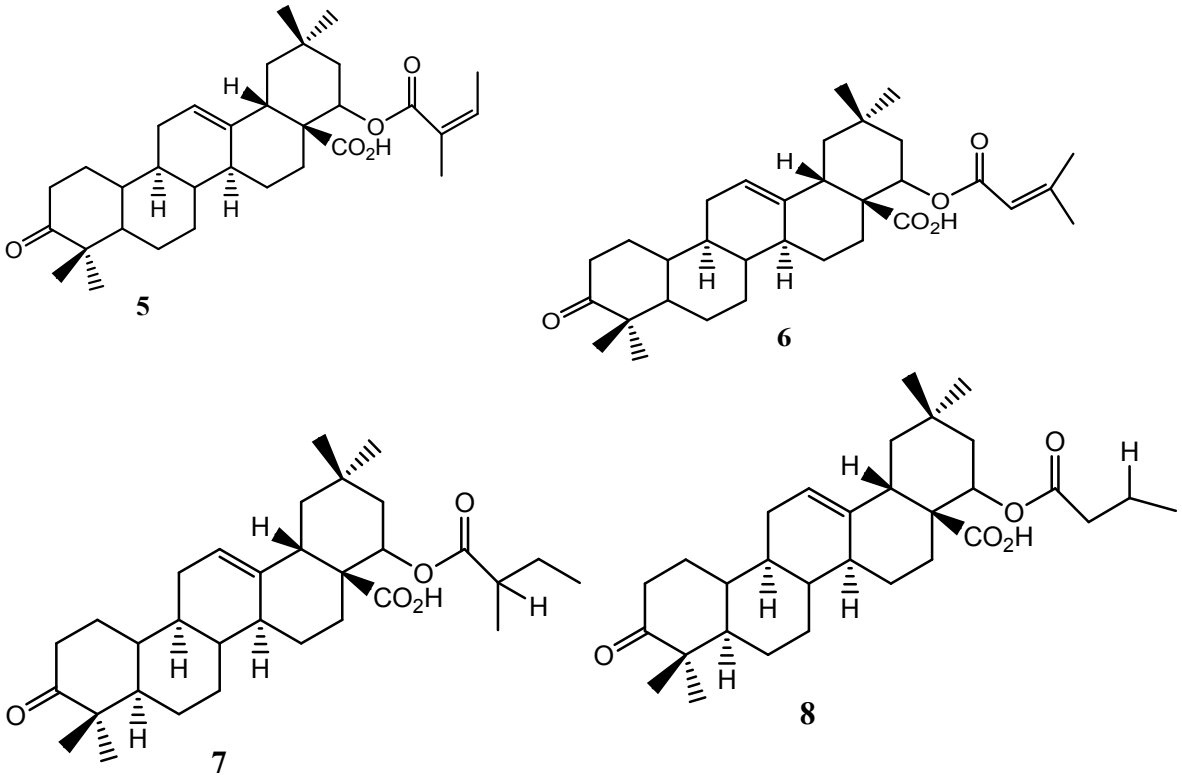
Lantana camara is a reservoir of several important bioactive molecules. A number of biological activities have been associated with various parts of *Lantana*, in folk medicine. Roots of *Lantana* plant are rich in oleanoic acid, hpatoprotective triterpenoid[26]. Translactone-containing triterpenes from *Lantana* leaves showed thrombin inhibitory activity and are lead compounds for drug discovery[32]. Pentacyclic trrierpenoids (the class of compounds to which lantadenes belong) are the focus of attention for drug research for anticancer, anti AIDS, antiinflammatory and antimicrobial activities[33]. It has been listed as one of the important medicinal plants of the world.

2.3. Compounds from *Lantana camara*

Reports showed that there are compounds that belong to different classes of compounds including alkaloids, terpenoids, phenolics, flavonoids, steroids, mono- and sesquiterpenes, triterpenes, iridoid glycosides, furanonaphthoquinones, flavonoids, phenylethanoides and glycosides[17, 34]. Phytochemical screening revealed that leaf, stem and root of *Lantana camara* contained tannin, catachin, saponin, steroids, alkaloids, phenol, anthroquinone, protein, several tri-terpenoids, flavonoids, alkaloids, glycosides and reducing sugar, which are mainly responsible for exerting diverse biological activities[35]. The isolation and identification of six novel compounds from *Lantana camara* from Brazil 1, 12-epoxycadina-3, 11-dienes, italicen-15-al (2,11-cycloacor-3-en-15-al), 6,10-epoxybisabolen-12-al and *epi*-abisabolone, *ar*-curcumen-15-al and its 10, 11-epoxides, methyl(E)-*trans*-a-bevgamota-2,10- dien-12-oate and its 2, 3-epoxide; and helifolen-12-al have been reported[36]. The essential oil of *Lantana camara* from Nigeria has *β*-caryophyllene as major compound together with α-humulene, sabinene, germacine D and cubebol. The essential oil of *Lantana camara* from South China was characterised by a high percentage of sesquiterpenes. The main components detected were germacnene D, *β*-caryophyllene *α*-humulene and germacrone B. The oxygenated monoterpene 1,8-cineole was reported in relatively high amounts compared to other monoterpenes. Essential oils of *Lantana* camara from India was reported to have germacrene D as the major compound in the leaves together with *γ*-elemene, *β*-caryophyllene, *β*-elemene, a-copaene, a-cadinene while in the flower oil *β*-clemene was a major compound together with germacrene D, α-copaene,*β*-caryophyllene and *γ*-elemene. *Lantana camara* leaf oil which is rich in sesquiterpes: *γ*-cadinene, *α*-selinene, *β*-gurjunene, eudesma-3, 11-diene, δ-cadinene and isolongifolene, constituting over half of the oil has been reported[37]. Extensive phytochemical studies on *Lantana* have led to the isolation of triterpenoids, flavonoids, iridoids, phenylpropanoids glycosine and verbascoside. The toxic effects of the species have been attributed to a series of pentacyclic triterpenes, of which lantadenes A and B are typical members[38].

2.3.1. Triterpenes

Triterpenes are a large class of compounds that include steroids and sterols. This class is present abundantly in plants and animals. They have a C_{30} carbon skeleton and most naturally occurring triterpenoids are biosynthesized from squalene. They also subdivided into some 20 groups, depending on their particular structures. Some triterpenoid compounds are found as saponin glycosides which refer to the attachment of various sugar molecules to the triterpene unit[39]. Recently many studies have been conducted to determine the biological activity of this class of compounds. Several triterpenoids have diverse pharmacological properties including antifungal, antibacterial, and antimutagenic activity[40]. The triterpenes are known to inhibit the action of multidrug resistance (MDR) protein, which leads to the failure of several potential anticancer agents [41]. Among triterpenes, lantadenes are pentacyclic triterpenoids of the *Lantana camara* belongs to the oleanane series, which considerd to have antibacterial, antiinflammatory, antiAIDS activity because of their toxicity and antitumor activity[42]. Literature report revealed that triterpenoids isolated from *Lantana camara* were; lantadene-A (**5**), lantadene-B (**6**), lantadene-C (**7**), lantadene-D (**8**), pentacyclic triterpenes like reduced lantadene (**9**), ursolate acetate (**10**), ursolic acid (**11**), lantic acid (**12**) and lantalonic acid (**13**) (Figure 3).[43]



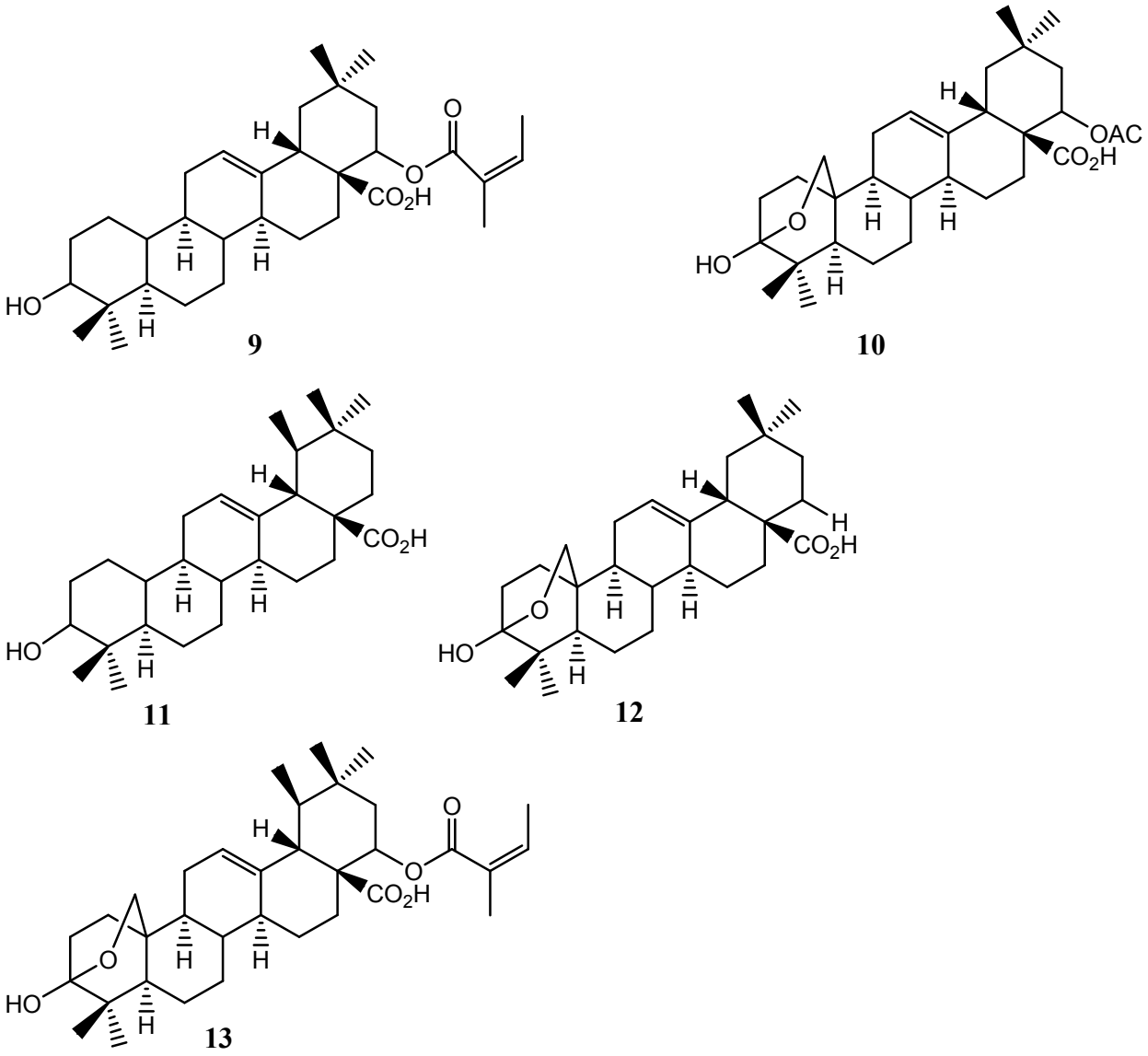


Figure 3. Pentacyclic triterpenes from *Lantana camara*[43]

The known triterpenoids icterogenin (**14**), betulonic acid (**15**), and betulinic acid (**16**), *β*-sitosterol 3-O-*β*-D-glucoside (**17**), and a mixture of campesterol (**18**), *β*-sitosterol (**19**) and stigmasterol (**20**) were also isolated from the stems of pink-flowering *Lantana camara* (Figure 4)[44].

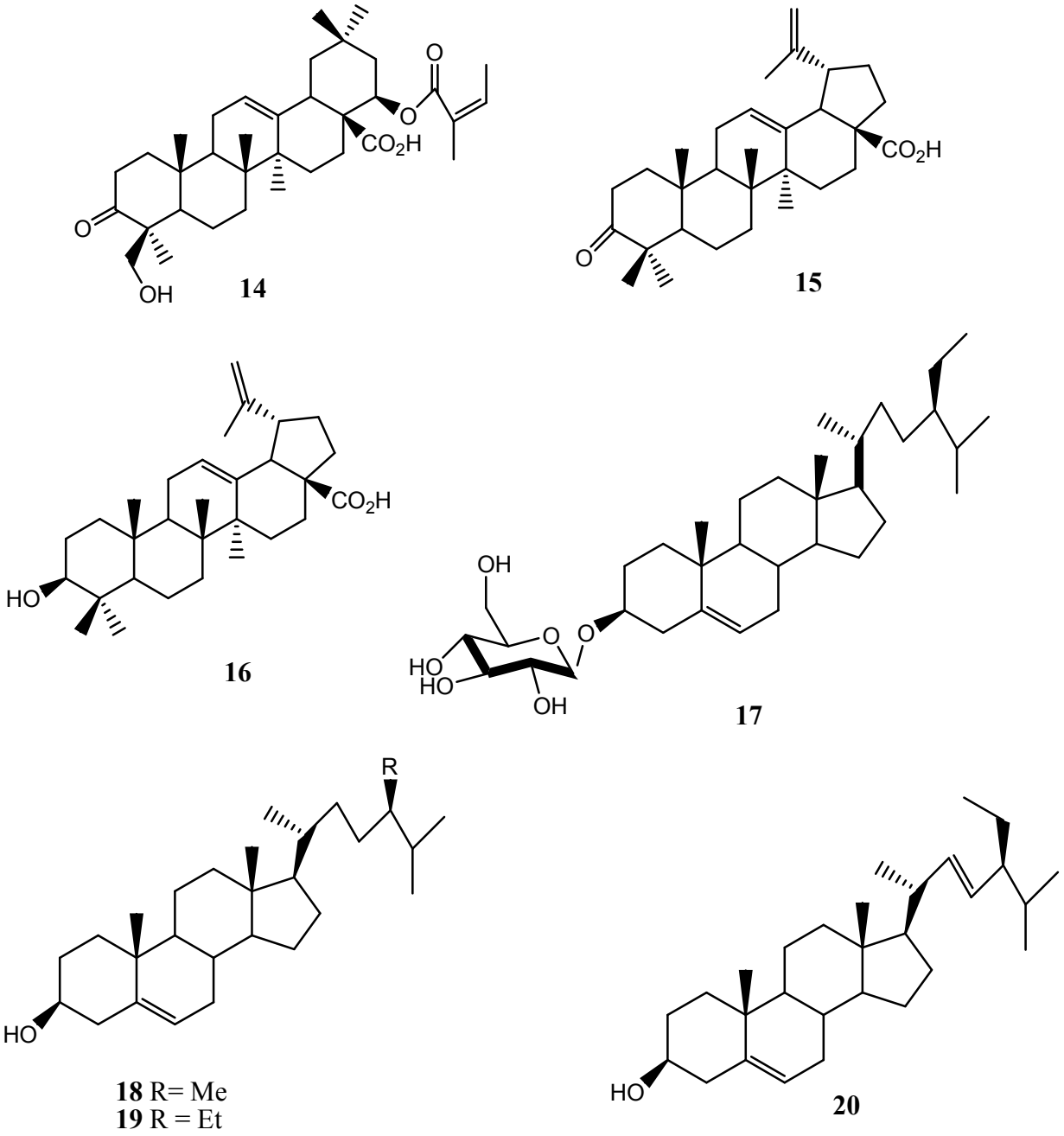


Figure 4. Triterpenoids from stem of pink flowering *Lantana camara*[44]

2.3.2. Flavonoids

Flavonoids are polyphenolic compounds which posses a C_{15} ($C_6C_3C_6$) framework. They contain Achroman ring (C-ring) with a second aromatic ring (B-ring) at the C-2, C-3, or C-4 position. The heterocyclic six-membered C-ring is sometimes replaced by a five-membered ring (e.g., aurones) or the acyclic form (chalcones). The oxidation state of C-ring is used to classify

flavonoids into different categories, of which typical examples are flavan-3-ols, flavanones, flavones and flavonols[45]. Literature report showed that the following flavonoids are isolated from *Lantana camara* leaves and flower. They were Hispidulin (**21**), 3-methoxy quercetin (**22**), 3, 7-dimethoxy quercetrin (**23**), 3, 7, 4-trimethoxy quercetin (**24**) and camaraside (**25**) (Figure 5)[46].

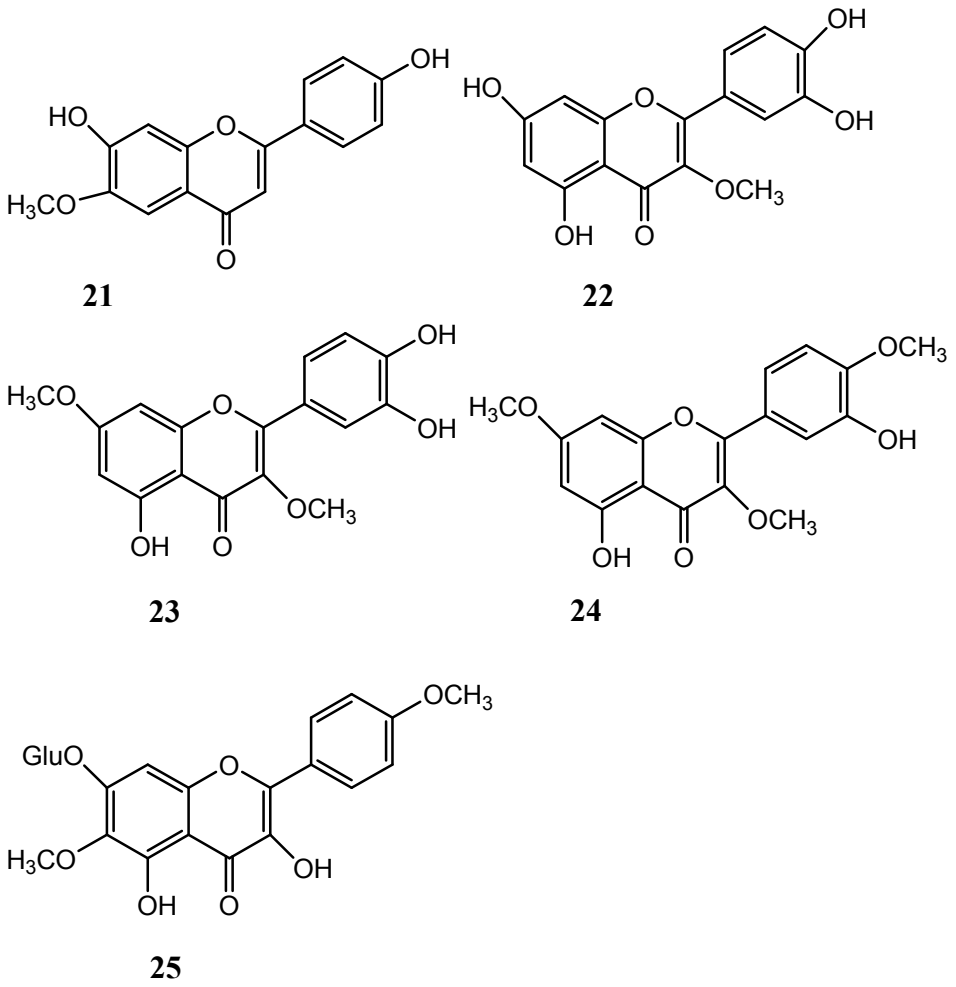


Figure 5. Flavonoids from *Lantana camara*[46]

2.3.3. Iridoid glycosides

Iridoid glycosides represent a large group of cyclopentano[*c*]pyran monoterpenoids which have been reported to have diverse biological activities including choleretic, purgative, liver protective, vasoconstrictive, antibacterial, analgesic, antitumor and antiinflammatory properties[47]. Literature reported that iridoid glycosides isolated from *Lantana camara* roots,

leaves and steams were theveside (**26**), theviridoside (**27**), gepriposide (**28**), 8-epiloganin (**29**), shanzhside mrthyl ester (**30**) and lamiridoside (**31**) (Figure 6)[47].

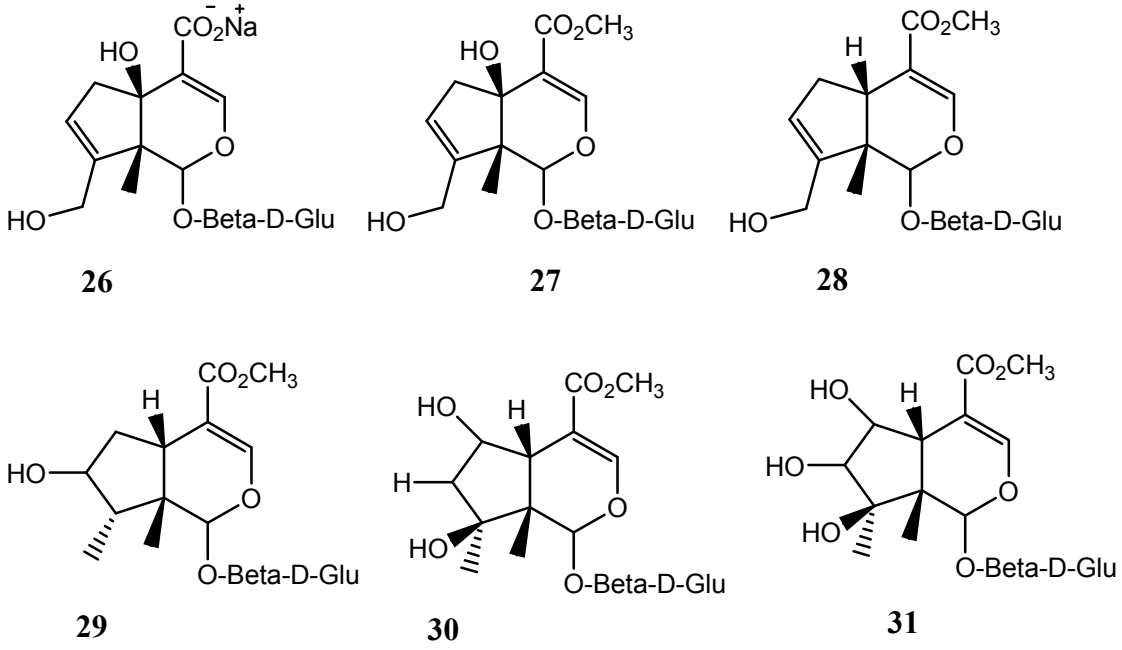


Figure 6. Iridoid glycosides from *Lantana camara*[47]

2.3.4. Phenolic compounds

Six phenolic compounds in a *Lantana camara* leaves extract were also identified by HPLC salicylic acid (**32**), gentisic acid (**33**), *β*-resorcylic acid (**34**), coumarin (**35**), ferulic acid (**36**), and 6- coumarin (**37**) (Figure 7)[48].

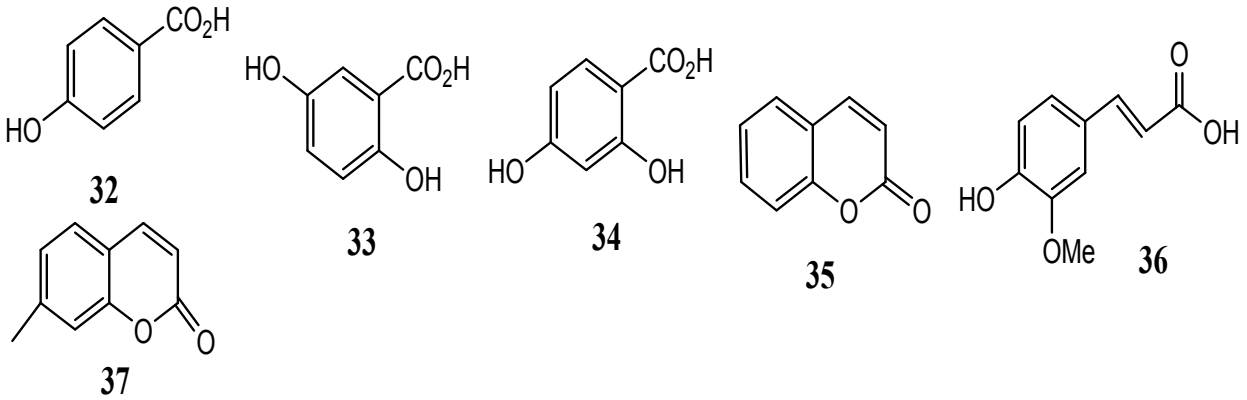


Figure 7. Phenolic compounds from *Lantana camara*[48]

3. Materials and Methods

3.1. Chemicals

Chemicals that were used for study include petroleum ether, chloroform, acetone and methanol for gradient extraction; petroleum ether and ethyl acetate for column elution; silica gel (60-120 mm mesh size), TLC plates and iodine for detection of spots on TLC were used. Dimethyl sulfoxide (DMSO), Mueller Hinton agar, nutrient broth and standard antibiotic gentamicine were used for antibacterial activity tests. All the chemicals and reagents used were analytical grade.

3.2. Apparatus and equipments

Apparatus such as rotary shaker (HY-5A Manoeuvre style vibrator) was used for extraction, rotary evaporator (Heidolph, UK) was used for evaporation of solvent, compound spots on TLC plates were detected using uvitec chamber (UV-254 & UV-365), glass columns for column chromatography. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 were recorded using Bruker advance 400 MHz spectrometer with TMS as internal standard, deuterated chloroform ($CDCl_3$) was used as a solvent in all spectroscopic analysis. An infrared (IR) spectrum (KBr) was obtained from Perkin-Elmer BX infrared spectrometer (400-4000 cm^{-1}).

3.3. Collection and preparation of plant material

Fresh fruit of *Lantana camara* which have mixture of pink, white and yellow flower taxa were collected from around Jimma University Institute of Technology, Jimma city, Oromia region in February 2014. Botanical identification was made by Dr.Kitessa Hundera (a botanist) and a specimen is deposited (voucher number 001) in the herbarium of the Department of Biology, Jimma University. The collected plant fruits were washed with distilled water and shade dried in laboratory at room temperature. The dried part was grounded with electrical grinder so as to enhance the penetration of solvents into cells of the plant powder.

3.4. Extraction of *Lantana camara* fruits

Air dried powdered plant fruits (750 g) was sequentially extracted with petroleum ether, chloroform, acetone and methanol (1300 mL each) to ensure that wide polarity range of compound were extracted. 750 g of fine powder was extracted twice with petroleum ether for 72 hours with continuous shaking. The extract was filtered and evaporated by means of a rotary evaporator. The residue obtained from filtration of the petroleum ether extract was then extracted

two times for 72 hours with continuous shaking with chloroform and the combined extracts were filtered and evaporated. The methanol and acetone extracts were prepared in the same way as the chloroform extract. The gradient extracts were filtered using fresh cotton plug and then using Whatman filter paper No.1. The solvent in each gradient extract was removed using rotary evaporator under reduced pressure and the resulting semidried mass of each extract were stored at 4 °C until used for experiments[49].

3.5. Preparation of test samples and bacterial strains for evaluation of antibacterial activity

Test solutions were prepared by dissolving 100 mg of each of crude extract in 1mL of dimethylsulfoxide (DMSO). Microorganisms used for evaluation of antibacterial activities of the crude plant extracts and isolated compounds were: *Staphylococcus aureus* OSM7346, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* DSMZ1117 and *Salmonella thyphimurium* ATCC13311. These strains were donated by the Department of Biology, Jimma University.

3.6. Preparation of fresh inoculums

Stock bacterial cultures were maintained at 4 °C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of bacterial cells from the stock cultures to test tubes of nutrient broth that were incubated without agitation for 24 hrs at 37 °C. A cell suspension of each organisms were freshly prepared by transferring isolated colonies selected from a 24 hrs agar plate in to a broth and the suspension turbidity adjusted to a 0.5 McFarland turbidity standard ($1x10^8$ CFU/ml) in sterile saline solution[50].

3.7. Antibacterial assay by disc diffusion method

About 100 µL of bacterial suspensions obtained above was spread over 90 mm Petri dishes containing Mueller-Hinton agar using a sterile cotton swab. Then six mm diameter sterile discs (Whatman No 3 paper) were placed on the surface of the inoculated agar in Petri dishes, and 20 µl each test solutions were applied onto the discs. The same volume of DMSO was used for negative control by using 6 mm diameter Whatman filter paper disc, and also standard gentamicine (30 µg) was used as positive control for comparison of antibacterial activity. After addition of test solutions on the discs, the extract was allowed to diffuse for 5-10 minutes and the plates were then kept in an incubatator at 37 °C for 24 hrs. The antibacterial activities were

evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler[51]. The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

3.8. Isolation and characterization of compounds

The crude chloroform extract of *Lantana camara* fruits was subjected to column chromatography over silica gel to isolate compounds. A glass column was packed with 100 g activated silica gel (60-120 mesh) slurry dissolved in petroleum ether. 5 g of the crude extract obtained from extraction was mixed with 7 g of silica gel and 10 mL of chloroform to obtain homogenous mixture. Then the solvent was allowed to evaporate, and the dry sample was applied into the column that was already packed with silica gel. Solvent system for elution of the column was determined after carrying out the TLC analysis of the extracts in various combinations of petroleum ether, chloroform, ethyl acetate, acetone and methanol among them petroleum ether: ethyl acetate (70:30) show distinct spots. The column was packed using the lower polar (100 % petroleum ether) solvent. Then the crude extract was applied on the column and eluted with increasing polarity of petroleum ethyl acetate mixture and fractions were collected. Each fractions was analyzed with TLC and visualized under UV light at 254 and 365 nm and then by exposure to iodine vapor. The fractions that showed the same TLC development profiles (color and Rf) were combined and concentrated to dryness under reduced pressure using rotary evaporator. The isolated pure compounds were then characterized by the various spectroscopic techniques namely, IR, ^{1}HNMR, ^{13}CNMR, DEPT-135 as well as comparison of their data with data reported in literatures. All spectroscopic analysis were carried out at the Department of Chemistry, Addis Ababa University.

4. Results and discussions

4.1. Sequential extraction of *Lantana camara* fruits

Extraction was carried out at room temperature under normal condition by maceration techniques. Dried fruits of *Lantana camara* (750 g) was powdered and subjected to extraction in rotary shaker apparatus at room temperature using petroleum ether, chloroform, acetone and methanol (successively) with solvent of increasing polarity for 72 hrs in each solvent with continuous shaking. Before extraction with the next solvent the marc was air dried to remove the traces of adhering solvent. The percentage yields of extracts obtained are given in Table 1.

Table 1. Percentage yields of *Lantana camara* fruits extract

Extractant	Mass extracted (g)	% Yield
Petroleum ether	6.72	0.896
Chloroform	5.15	0.687
Acetone	15.77	2.103
Methanol	12.73	1.697

In the present study, the percentage yield of the extract of *Lantana camara* fruits was obtained from acetone extract (2.103 %), followed by methanol (1.697 %), petroleum ether (0.896 %) and chloroform (0.687%) (Table 1).

4.2. Evaluation of antibacterial activities of crude extracts from *Lantana camara* fruits

The inhibitory activity of the four gradient extracts of *Lantana camara* fruits against some human pathogenic bacteria was presented in Table 2. In the present work, *in vitro* studies indicated that the *Lantana camara* different solvent extract inhibited bacterial growth but their effectiveness varied. Among the four different extracts tested, chloroform extracts has shown superior antibacterial activity followed by acetone and petroleum ether extracts, whereas no significant antibacterial activity was observed in methanol extracts. The control DMSO did not inhibit any of the bacteria species used in the experiment. The standard gentamicine showed maximum antibacterial activity compared to four extracts. The chloroform extract exhibited antibacterial activity with zone of inhibition ranging from 12 mm to 16 mm at 100 mg/mL

concentration depending upon bacterial species. The most susceptible organism in the present investigation was *Pseudomonas aeruginosa* followed by *Salmonella thyphimurium* and *Staphylococcus aureus*, whereas *Escherichia coli* were found to be most resistant bacteria against all the extracts tested (Appendix 17). The effectiveness of the extracts varies with its type and the bacteria species used in the study. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures. It is the ability of the active principle of the extracts that disrupt the permeability barrier of cell membrane structures and thus inhibit the bacterial growth [52].

Table 2. Antibacterial activity of different solvent extracts of *Lantana camara* fruit against bacterial strains.

Bacteria strain	Conc. mg/ml	Zone of inhibition in (mm)					
		Petroleum extract	Chloroform extract	Acetone extract	Methanol extract	Gentamicine	DMSO
Escherichia coli	100	9	12	12	8	22	NI
Staphylococcus aureus	100	10	13	10	9	22	NI
Salmonella thyphimurium	100	12	14	13	11	23	NI
Pseudomonas aeruginosa	100	12	16	14	12	24	NI

Note; NI = not inhibitory

The results were consistent with previous reports also described antibacterial activities of *Lantana camara* leaves chloroform extract that gave 15 mm, 13 mm and 12 mm zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* respectively, whereas petroleum extract gave 13 mm, 11 mm, and 9 mm zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* respectively[53]. Therefore, the crude chloroform extract of *Lantana camara* fruit was selected as the best candidate for chromatographic isolation of compounds.

4.3. Isolation of compounds by column chromatography

The crude 5 g of chloroform extract was adsorbed onto 7 g of silica gel that subsequently loaded into glass column packed with 100 g of silica gel (60-120 mesh). The column was initially eluted with 100 % petroleum ether and then followed with petroleum ether and ethyl acetate mixture in different combination with increasing polarity (in the ratio 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 up to 0:100 %). A total of 253 fractions each with 15 mL were collected in test tube and TLC analysis was done. According to TLC profiles, these fractions were reduced to 25 fractions. The developed spots on TLC plates were visualized under UV light at 254 and 365 nm and then by exposure to iodine vapor. Among reduced 25 fractions, fraction 9 (27 mg) eluted with petroleum ether ethyl acetate (85:15) resulted single spot on TLC and showed Rf value of 0.65 (80:20 petroleum ether ethyl acetate). After removing the solvent a white waxy powder with characteristic odor was resulted. The compound was designated as compound **LC-1**. Fraction 14 eluted with petroleum ether ethyl acetate (70:30) also showed single spot on TLC. Concentration of this fraction gave 30 mg of black greenish amorphous solid. Its Rf value was determined as 0.58 in petroleum ether ethyl acetate (65:35). This compound was labeled as compound **LC-2**. Fraction-15 was rechromatographed on silica gel eluting with petroleum ether ethyl acetate (70:30) and then 12 fractions (15A-15L) were collected each with 20 mL. Fractions 15A-15E was combined to afford 15 mg brown amorphous solid and its Rf value was determined as 0.52 in petroleum ether ethyl acetate (65:35). The compound was labeled as **LC-3**. Fraction 16 eluted with petroleum ether ethyl acetate (70:30) was also rechromatographed on silica gel eluting with petroleum ether ethyl acetate (65:35) yielded fractions 16A-16J each with 20 mL. Fractions 16A-16E were combined and concentrated to afford 23 mg pale yellow oily compound and its Rf value was determined to be 0.45 in petroleum ether ethyl acetate (60:40). The compound was labeled as **LC-4**. Similarly, fraction 3 eluted with petroleum ether ethyl acetate (90:10) was rechromatographed on silica gel eluting with petroleum ether ethyl acetate (80:20) were collected fractions 3A-3J each with 20 mL. Among this fractions 3G-3J showed single spot on TLC and its Rf value was determined as 0.72 in petroleum ether ethyl acetate (85:15). After removing the solvent yellowish oily compound was obtained. The compound was labeled as **LC-5** (18 mg) (Figure 8).

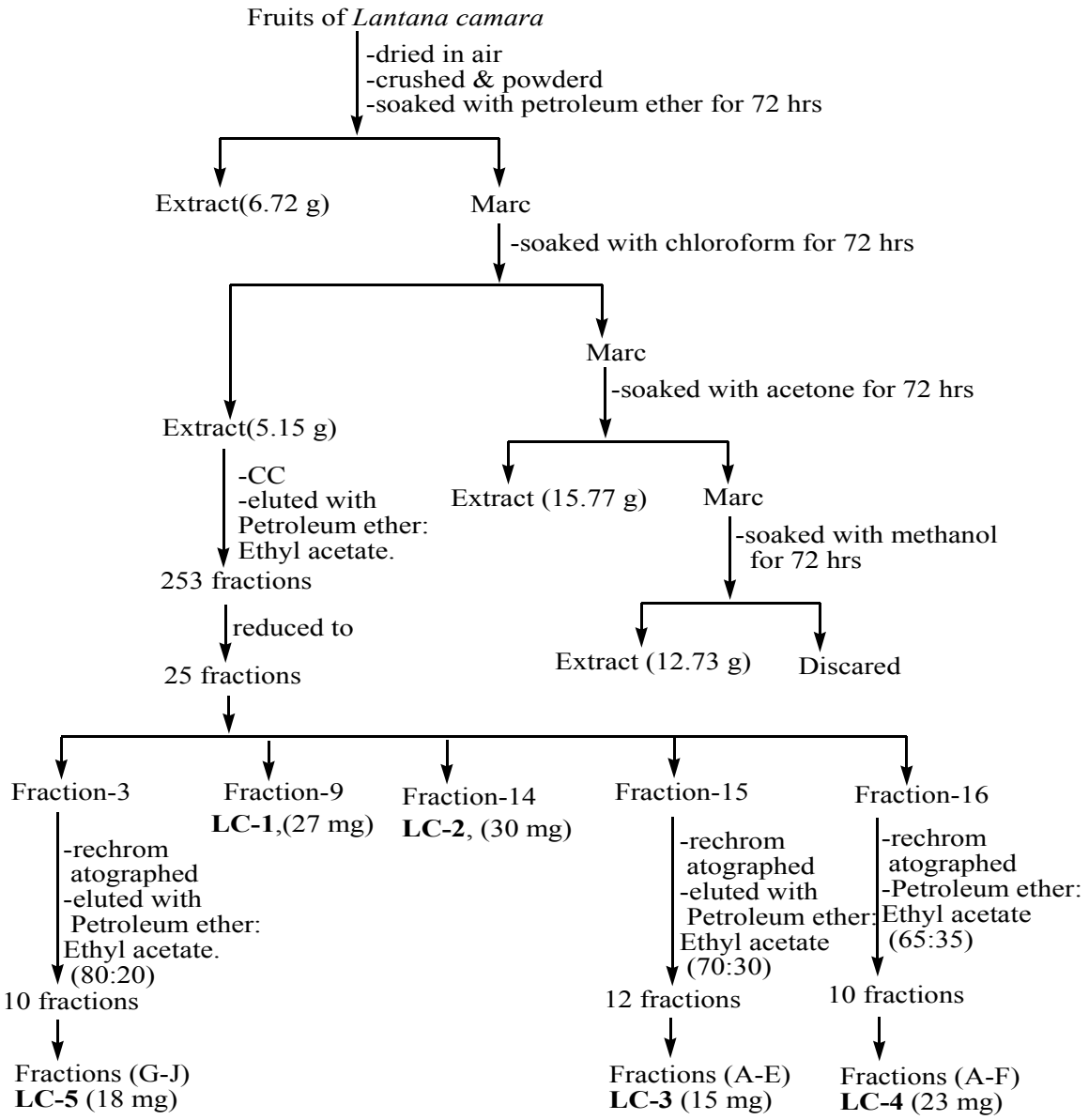


Figure 8. A scheme showing isolation and fractionation of compounds from *Lantana camara* fruit chloroform extracts.

4.4. Structural elucidation of the isolated compounds

The structure of the compounds (**LC-1**, **LC-4** and **LC-5**) isolated from chloroform extract of *Lantana camara* were discussed below. The compounds were characterized using spectroscopic techniques (NMR and IR spectroscopic techniques). The chemical shifts were given in ppm (δ) and were referenced relative to $CDCl_3$ (δ 7.28 and 76-77 ppm for ^1H and ^{13}C-NMR, respectively) spectrometer and the chemical shifts were expressed in (ppm) values with trimethylsilane as an

internal reference. The IR spectra were recorded on a Bruker FT-IR spectrometer. The structural elucidation was done by comparing the observed spectra with the reported data of those compounds in the literature.

4.4.1. Structural elucidation of compound LC-1

Analysis of IR (KBr) spectrum of **LC-1** (Appendix 1) indicated the presence of OH stretching, CH-stretching and C=C stretching. The absence of a doublet band at/near 2800 and 2700 cm^{-1} also indicated that the compound has no aldehydic functional group. The band assigned at 3425 cm^{-1} due to OH stretching frequency of the compound. The band at 2931cm^{-1} shows the presence of –CH stretching frequency (C-H str. of CH_3). The C=C stretching shows at 1680 cm^{-1} and peak at 1464 cm^{-1} (C-H deformation of gem dimethyl) and the bands ranging from 1191- 660 cm^{-1} are characteristic bands of steroids. Thus, the IR data indicated the compound is an alcohol with C=C bond in its chain (Figure 9).

The ^{1}H-NMR of this compound (Appendix 2) exhibited methyl signals at 0.69 ppm (H-18), 0.82 ppm (H-26), 0.84 ppm (H-27), 0.87 ppm (H-29), 1.02 ppm (H-19), and 0.93 ppm (H-21). These peaks correspond to the H-atoms attached to the external CH_3 groups on the *β*-sitosterol. This compound has revealed one proton multiplet at 3.55 ppm (1H, C-3H) the position indicated the presence of a hydroxymethine group and multiplicity of the steroid nucleus. The proton at 5.36 ppm (1H, C-6H) belonged to olefinic hydrogen was evident for steroidal skeleton (Figure 9). The ^{13}C-NMR and DEPT spectrum gave 29 signals; six methyl, eleven methylene, nine methine and three quaternary carbons. The ^{13}C-NMR spectrum (Appendix 3) showed peaks at two downfield signals at δ 140.7 and 121.8 belong to the endocyclic carbon-carbon double bond C-5 and C-6, respectively. A significant peak at δ 71.9 represents the C-3 that is bonded to the hydroxyl group. According to the DEPT spectra, the peaks at δ 42.2, 39.8, 37.3, 34.0, 31.6, 31.5, 23.1, 28.4, 26.1, 24.3, and 21.1 represented the methylene groups of C-4, C-12, C-1, C-22, C-7, C-2, C-28, C-16, C-23, C-15, and C-11, respectively. The absence of peaks at 140.7 (C-5), 36.5 (C-10) and 42.4 (C-13) ppm in the DEPT-135 spectrum which observed in the^{13}C-NMR spectrum also confirmed the presence of quaternary carbon atoms in **LC-1** (Appendix 4). This information suggested the compound to be sterol with four rings occurring in the molecule. Based on spectroscopic data and comparison with the reported literature[54], the compound was characterized as *β*-sitosterol (Figure 9). The NMR data of compound **LC-1** and that of *β*-sitosterol are given in Table 3.

Table 3. ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of **LC-1** with reported data of *β*-sitosterol

Position	^{13}C-NMR of **LC-1**	Reported of *β*-sitosterol[54]	DEPT-135 of **LC-1**	^{1}H-NMR of **LC-1**	Reported ^{1}H-NMR of *β*-sitosterol[54]	Remark
1	37.3	37.3	37.3			CH_2
2	31.5	31.7	31.6			CH_2
3	71.9	71.8	71.9	3.55	3.53	CH
4	42.2	42.2	42.2			CH_2
5	140.7	140.8				C
6	121.8	121.7	121.8	5.36	5.36	CH
7	31.6	31.8	31.6			CH_2
8	31.9	31.9	31.9			CH
9	50.1	50.1	50.1			CH
10	36.5	36.5				C
11	21.1	21.1	21.1			CH_2
12	39.9	39.8	39.8			CH_2
13	42.3	42.3				C
14	56.8	56.9	56.8			CH
15	24.3	24.4	24.3			CH_2
16	28.3	28.2	28.3			CH_2
17	56.1	56.1	56.1			CH
18	11.9	11.9	11.9	0.69	0.69	CH_3
19	19.4	19.4	19.4	1.02	1.03	CH_3
20	36.2	36.2	36.2			CH
21	18.8	18.8	18.8	0.93	0.93	CH_3
22	34.0	34.0	33.9			CH_2
23	26.1	26.1	26.1			CH_2
24	45.9	45.9	45.8			CH
25	29.7	29.1	29.7			CH
26	19.8	19.8	19.9	0.82	0.83	CH_3
27	19.0	19.0	19.0	0.84	0.85	CH_3
28	23.1	23.1	23.1			CH_2
29	12.0	12.1	12.0	0.86	0.87	CH_3

β-sitosterol is a natural micro-nutrient which is found in the cells and membranes of all oil producing plants, fruit, vegetables, grains, seeds and trees. It has been proven to be a safe, natural and effective nutritional supplement and has shown amazing potential benefits in many diverse applications[55]. Earlier experimental studies have shown it is important bioactive component exhibiting various pharmacological properties such as antiinflammatory, antipyretic,

antiarthritic, antiulcer, insulin releasing, antidiabetic, antioxidant and antistress agent[55]. Literature survey also revealed that *β*-sitosterol has been isolated from various medicinal plants such as stem of *Sida rhombifolia*[56], *Lagenaria siceraria* fruits[57], aerial parts of *Etlingera brevilabrum*[54], and it has also been previously isolated from different parts of *Lantana camara* plants such as leaves and steams of the yellow flowering taxa[58, 59], from the stems of pink-flowering taxa[14], and from its roots[60].

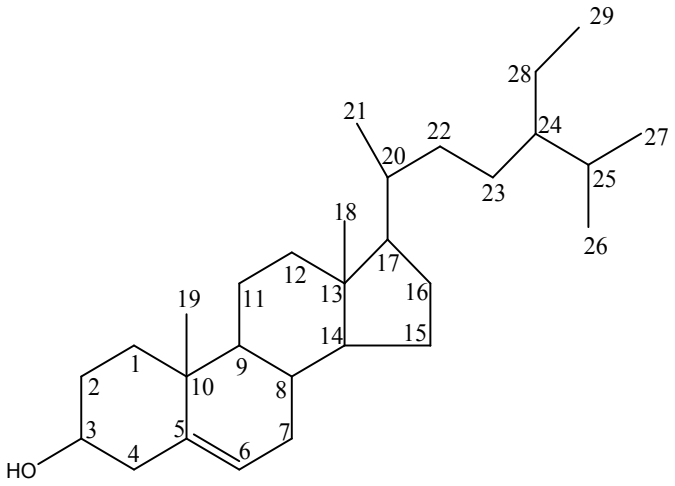


Figure 9. *β*-sitosterol (**LC-1**)

4.4.2. Structural elucidation of compound LC-4

Analysis of IR (KBr) spectrum (Appendix 5) clearly showed strong adsorption bands at (1731 and 1273 cm^{-1}) that correspond to the carbonyl ester indicated the presence of ester moiety and aromatic (1663, 1498 and 743 cm^{-1}). The band at 2929 cm^{-1} showed the presence of -CH stretching frequency (C-H str. of CH_2 and CH_3). ^{1}H-NMR (Appendix 6) showed single peak at δ 7.28 observed due to solvent chloroform-d ($CDCl_3$) and the presence of two sets of aromatic protons in the molecule which has suggested that the compound must have an ortho disubstituted benzene ring bearing the same substituent in both positions. Downfield shifted multiplets for aromatic protons are detected at δ 7.72-7.73 and (H-2 and H-2') and 7.56 [H-1 and H-1']. The ^{1}H-NMR spectrum also confirmed the presence of oxy-methylene proton at δ 4.22-4.25 (H-5 and H-5'), four terminal methyl protons at δ 0.86 (H-10 and H-10') and 0.94 (H-12 and H-12'). The observed ^{1}H-NMR spectral data (Table 4) of compound **LC-4** was found to be consistent to that of di-(2-ethylhexyl) phthalate reported in literature[61]. The ^{13}C-NMR spectrum (Appendix 7) and DEPT-135 spectra (Appendix 8) displayed twelve carbon resonances composed with a carbonyl

ester carbon at δ 167.8 (C-4 and C-4'), three aromatic carbons including quaternary carbons at δ 128.8 (C-2 and C-2'), 130.9 (C-1 and C-1') and 132.5 (C-3 and C-3'), one another methine at δ 38.8 (C-6 and C-6'), five methylene carbons at δ 23.0 (C-9 and C-9'), 23.8 (C-11 and C-11'), 29.0 (C-8 and C-8'), 30.4 (C-7 and C-7') and 68.2 (C-5 and C-5') and two methyl carbons at δ 11.0 (C-12 and C-12'), and 14.1 (C-10 and C-10'). Oxygenated methylene indicated the direct attachment of carbonyl group to benzene ring and two different methyl groups indicated that remaining side chain is branched octyl (Figure 10). By comparison of ^{1}H and ^{13}C-NMR data to those published in literature[61] compound **LC-4** was identified as di-(2-ethylhexyl) phthalate. The NMR data of **LC-4** and that of di-(2-ethylhexyl) phthalate are given in Table 4. This bioactive compound was earlier isolated from the methanol extract of *Lantana camara* flower and leaf [62], and also isolated from medicinal plant such as unripe fruits of *Nauclea latifolia* that exhibited better antibacterial efficacy against some Gram positive *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains[61], from the seeds of *Ricinus communis* which have antibacterial effect on a number of bacteria strains[63], from *Cyperus rotundus* which showed that good antiinflammatory activity[64], from *Ehretia laevis* which have anti microbial, entomological as well as anti-inflammatory activities[65].

Table 4. ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of **LC-4** with reported data of di-(2-ethylhexyl) phthalate[61]

Position	^{13}C-NMR of **LC-4**	Reported data of di-(2-ethylhexyl) phthalate [61]	DEPT-135 of **LC-4**	DEPT-135 of di-(2-ethylhexyl phthalate[61]	^{1}H-NMR of **LC-4**	Reported ^{1}H-NMR data of di-(2-ethylhexyl) phthalate [61]	Remark
1,1’	130.9	130.9	130.9	130.9	7.56	7.55-7.57	CH
2,2’	128.8	128.8	128.8	128.8	7.72-7.73	7.70-7.74	CH
3,3’	132.5	132.5	-	-	-	-	C
4,4’	167.8	167.8	-	-	-	-	C
5,5’	68.2	68.2	68.2	68.2	4.22-4.25	4.20-4.28	CH_2
6,6’	38.9	38.7	38.8	38.7	1.69-1.71	1.63-1.76	CH
7,7’	30.4	31.4	30.4	30.4	1.34	1.30-1.35	CH_2
8,8’	29.0	29.7	29.0	28.9	1.28	1.29	CH_2
9,9’	23.0	23.0	23.0	23.0	1.28	1.29	CH_2
10,10’	14.1	14.0	14.1	14.1	0.86	0.89-1.00	CH_3
11,11’	23.8	23.8	23.8	23.8	1.43	1.40-1.47	CH_2
12,12’	11.0	11.0	11.0	11.0	0.94	0.89-1.00	CH_3

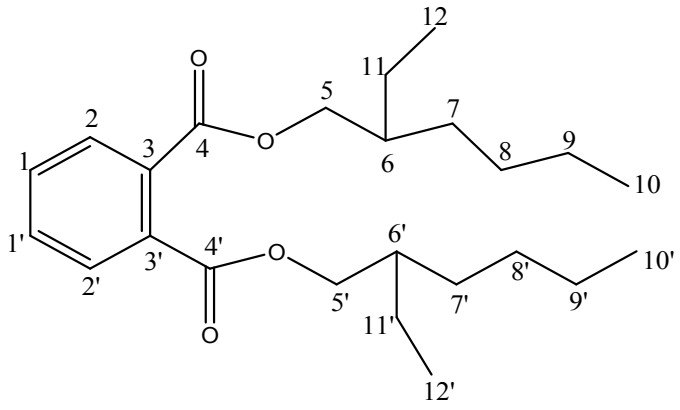


Figure 10. Di-(2-ethylhexyl) phthalate (**LC-4**)

4.4.3. Structural elucidation of compound LC-5

Analysis of IR spectrum of **LC-5** (Appendix 9) indicated that the carbonyl group stretching band at 1731 cm^{-1} indicated that the compound is most likely an ester. The broad band at 1298 cm^{-1} also indicates the C-O stretching vibration of an ester. The absorption band observed at 3030cm^{-1} showed the presence of the C-H stretching of the olefin. The absorption band at 1710 cm^{-1} and band at 2918 cm^{-1} showed the presence of the olefinic C=C stretching and C-H stretching of the methylene groups, respectively. ^{1}H-NMR spectrum of compound **LC-5** (Appendix 10) showed peak at δ 0.90 indicates protons of methyl groups. Signals at δ 4.31 and 4.10 indicated protons that were close with the atom oxygen in the glycerol chain, the peak at δ 5.36 indicated methynic group of glyceryl (CHO) proton and a peak at δ 5.38 indicates the presence of olefinic protons. Other present signal at δ 2.79 indicates for methylenic group between olefinic protons; peak at δ 2.38 indicates the presence of methylenic groups in a position with respect to carbonylic group; peak at δ 2.06 indicates the presence of methylenic group in both sides of olefinic protons; peak at δ 1.69 indicates for methylenic group in *β*-position with respect to carbonylic group and peaks at δ 1.28 to 1.32 indicate for methylenic groups in triglyceride chain. The observed IR and ^{1}H-NMR data were found to be consistent with the reported data of trilinolein (Figure 11)[66].

The ^{13}C-NMR spectrum of compound **LC-5** (Appendix 11) contains the resonance of carbons from the triglyceride fraction that grouped in four sets of signals, carbonyl carbons resonating from 172.9 to 173.3 ppm, unsaturated carbons in the range from 127.9 to 130.2 ppm, glycerol backbone carbons from 62.1 to 68.9 ppm and aliphatic carbons from 14.1 to 34.1 ppm. The terminal methyl carbon(C-18) was found at δ 14.1 ppm, methylene group indicated at 22.7 (C-17), methylenic acylic chains at δ 24.7 (C-3) indicated in β-position with respect to carbonylic group; peak observed at δ 25.7 (C-11) indicate methylenic group between olefinic protons of linoleyl chains. Signals observed in the range of 29.1-29.7 indicate the methylene ($–CH_2$) in triglyceride chain; the peaks at δ 173.3 and 172.9 indicated quaternary carbon atoms of ester carbonyl group; the peaks observed at δ 130.0 (C-9), δ 128.1 (C-10), δ 127.9 (C-12) and δ 130.2 (C-13) indicated C=C bonds. The DEPT-135 spectrum (appendix 12) also confirmed the presence of methyl carbon at δ14.1, methylene carbon at δ 22.7 and 62.1, and oxygenated methine carbon at δ 68.9, and olefinic methine carbons at δ 127.9–130.2. The absence of peaks at δ 172.9 and 172.3 in the DEPT-135 spectra, which were observed in the ^{13}C-NMR spectra, also confirmed quaternary carbon atoms of ester carbonyl group (Table 6). Based on spectroscopic

data (IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135) and comparison with the reported literature, the compound was characterized as triglyceride trilinolein[66, 67] (Figure 11). The NMR data of **LC-5** and that of trilinolein were given in (Table 5). Previous literature reported that four triglycerides (1,2,3-triolein, 1,2, 3-trilinolein, 1,3-dilinoleoyl-2-olein, and 1,3-dioleoyl-2-linolein) have been reported from different medicinal plants such as *Moringa olifera*[68], *Moringa stenopetala* (1,3-dilinoleoyl-2-olein, and 1,3-dioleoyl-2-linolein)[69], *Cinnamomum cebuense* (1,2, 3-trilinolein)[66], *Dirca palustris* (1,2, 3-trilinolein, 1,3-dilinoleoyl-2-olein, and 1,3-dioleoyl-2-linolein)[70].

Table 5. ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of **LC-5** with reported data of trilinolein

Position	^{13}C-NMR of **LC-5**	Reported of trilinolein[66, 67]	DEPT-135 of **LC-5**	^{1}H-NMR data of **LC-5**	Reported ^{1}H-NMR of trilinolein[66,67]	Remark
1'a 1'b	62.1 62.1	62.1 62.1	62.1 62.1	4.31 4.10	4.28 4.12	CH_2
2'	68.9	68.9	68.9	5.36	5.32	CH
3'a 3'b	62.1 62.1	62.1 62.1	62.1 62.1	4.31 4.10	4.28 4.12	CH_2
1	172.9,173.3	172.8, 173.3	-	-	-	C
2	34.1	34.1	34.1	2.38	2.31	CH_2
3	24.7	24.8	24.7	1.65	1.35	CH_2
4	29.1	29.1	29.1	1.28	1.25	CH_2
5	29.3	29.3	29.3	1.28	1.25	CH_2
6	29.1	29.1	29.1	1.28	1.25	CH_2
7	29.7	29.6	29.69	1.32	1.30	CH_2
8	29.2	29.2	29.2	2.06	2.03	CH_2
9	130.0	130.0	130.0	5.38	5.33	CH
10	128.1	128.1	128.1	5.38	5.33	CH
11	25.7	25.6	25.7	2.79	2.75	CH_2
12	127.9	127.9	127.9	5.38	5.33	CH
13	130.2	130.2	130.2	5.38	5.33	CH
14	27.2	27.2	27.2	2.06	2.03	CH_2
15	29.4	29.4	29.4	1.32	1.30	CH_2
16	32.0	31.5	32.0	1.28	1.25	CH_2
17	22.7	22.6	22.7	1.28	1.25	CH_2
18	14.1	14.1	14.1	0.90	0.87	CH_3

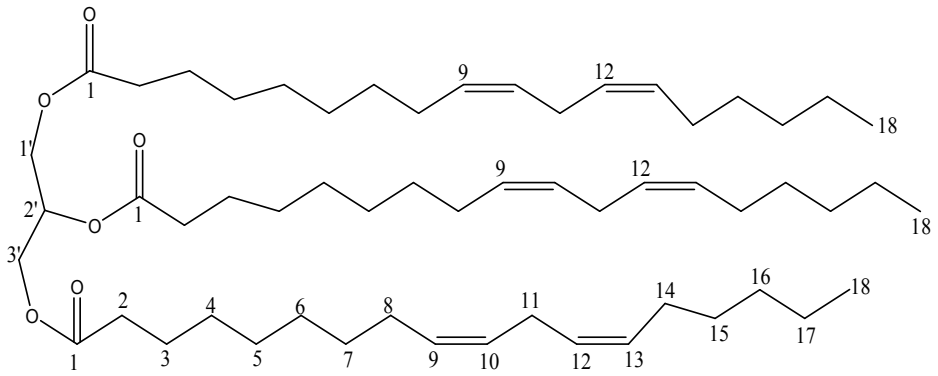


Figure 11. Trilinolein (**LC-5**)

4.4.4. Partial characterization of compound LC-2

The compound **LC-2** (30 mg) was obtained as black greenish amorphous solid. Its Rf value was determined as 0.58 in petroleum ether ethyl acetate (65:35). In the IR spectrum of the compound **LC-2** (Appendix 13), the absorption band at 3401 cm^{-1} showed the stretching of OH that indicated the presence of the hydroxyl group. The absorption band at 2929 cm^{-1} showed the C-H stretching of the methyl group. A broader band was also observed at 1703 cm^{-1} showed the presence of the olefinic C=C stretching and band at 1462 may probably be due to aromatic carbon in the compound. The presence of an aromatic group in compound **LC-2** is also supported by its ^{1}H-NMR spectrum (Appendix 14). The ^{1}H NMR spectrum depicts different separated regions of protons, one in the aromatic region at δ 7.56- 7.74, olefinic region at δ 4.22-5.60, methoxy region at δ 3.30-3.39 and the other in the aliphatic region at δ 0.80- 2.95. The ^{13}C-NMR spectrum of compound **LC-2** (Appendix 15) also strengthens the fact that there are different chemical shift regions, one from δ 129.1- 133.0 which is for the aromatic group, δ 138.1 for olefinic carbon, from δ 67.2-73.1 for methoxy carbons and the other from δ 11.0-56.4 which is for the aliphatic group. On the other hand, the chemical shift values in the range of 11.0-56.4 ppm indicated presence of methyl ($-CH_3$), methylene ($-CH_2$) and methine (-CH) carbons. Analysis of IR and NMR spectra of **LC-2** indicated that the compound exhibited hydroxyl carbon of alcohol and aromatic ring carbon, but our literature survey does not help us to determine the structure of compound **LC-2** by relating the experimental data of compound **LC-2** with already published data in literature review section. In addition, due to lack of 2D-NMR and MS spectroscopic data, the structure of the compound **LC-2** was subjected to partial characterization.

4.5. Evaluation of antibacterial activities of the isolated compounds

The antibacterial activities of five isolated compounds (**LC-1**, **LC-2**, **LC-3**, **LC-4** and **LC-5**) were carried out using four bacterial species: *Pseudomonas aeruginosa, Salmonella thyphimurium, Staphylococcus aureus* and *Escherichia coli* by disc diffusion method. The growth inhibition zones (in mm) of the compounds are given in Table 6. The result indicated that **LC-4** (di-(2-ethylhexyl) phthalate) showed the maximum antibacterial activity against *Pseudomonas aeruginosa* 26 mm (it was higher than the standard antibiotic gentamicine, which showed 25 mm of inhibitory activity)*, Salmonella thyphimurium* 24 mm*, Staphylococcus aureus* 22 mm and *Escherichia coli* 20 mm. The results of this study are in agreement with reports of made in other studies which (di-(2-ethylhexyl) phthalate) isolated from different plants showed a better broad spectrum of antibacterial activity against both Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Sarcina lutea*) and Gram negative (*Escherchia coli*, *Shigella shiga* and *Shigella dysenteriae*) bacteria, with inhibition zones in the range of 13-24 mm[61, 71].

Similarly, **LC-5** (trilinolien) showed maximum antibacterial activity against *Pseudomonas aeruginosa* 21 mm, 20 mm against *Escherichia coli*, 19 mm against *Staphylococcus aureus,* and 11 mm against *Salmonella thyphimurium*. And **LC-1** (*β*-sitosterol) showed the maximum inhibitory activity 24 mm against *Pseudomonas aeruginosa,* 19 mm against *Staphylococcus aureus,* 17 mm against *Salmonella thyphimurium,* and 14 mm against *Escherichia coli*. Literature reported that *β*-sitoterol isolated from *Momordica charantia* plants showed the inhibition zone recorded 14 mm (*Escherichia coli*), 13 mm (*Staphylococcus aureus*), and 11 mm (*Pseudomonas aeruginosa)*[55]. Similar results were reported that *β*-sitoterol isolated from different plants showed good to moderate antibacterial activity against various Gram-positive and Gram-negative bacteria[72]. Thus these results prove the antibacterial potential of β-sitosterol isolated from the *Lantana camara* fruits have high antibacterial activity and this provide additional support for the use of the fruits of this plant as traditional medicine. Among the five isolated compounds **LC-3** showed the least activity with tested four bacteria strains that means 19 mm against *Pseudomonas aeruginosa,* 18 mm against *Staphylococcus aureus,* and 15 mm against *Salmonella thyphimurium* and 14 mm against *Escherichia coli.* Whereas the standard antibiotics gentamicine showed the inhibitory activity against *Pseudomonas aeruginosa* (25 mm), *Salmonella typhimurium* (22 mm), 21 mm against *Staphylococcus aureus* and 20 mm against *Escherichia coli*. The isolated compounds displayed moderate to good antibacterial

properties on the tested bacteria in which compound **LC-4** showed comparatively higher activity than other compounds. The most susceptible organism was *Pseudomonas aeruginosa* where as *Escherichia coli* was found to be most resistant bacteria against all the isolated compounds (Appendix 18). The overall results of this study is expected to provide evidence that *Lantana camara* fruits extract as well as the isolated compounds exhibit antibacterial activity for both Gram negative and Gram positive pathogens. Thus, the observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of the plant for the treatment of different bacterial infections.

Table 6. Antibacterial activity of isolated compounds in disc diffusion methods

Bacteria strain	Diameter of zone in mm						
	LC-1	**LC-2**	**LC-3**	**LC-4**	**LC-5**	Gentamicine	DMSO
Escherichia coli	14	19	14	20	20	22	NI
Staphylococcus aureus	19	17	20	22	19	23	NI
Salmonella thyphimurium	17	20	15	25	18	24	NI
Pseudomonas aeruginosa	24	22	20	26	21	25	NI

Note; NI = not inhibitory

5. Conclusions and recommendations

Based on our results, it can be concluded that *Lantana camara* fruits contain bioactive compound that are effective against the tested bacteria strains. The crude extracts were prepared from sequential solvent extraction of petroleum ether, chloroform, acetone and methanol extracts. Column chromatographic analysis of the chloroform extract has been carried out using petroleum ether and ethyl acetate combinations as eluent. Three compounds *β*-sitostrol (**LC-1**), di-2-ethylhexyl phthalate (**LC-4**) and trilinolien (**LC-5**) were isolated and characterized by using spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135) and by comparing the observed spectral data with literature reports. The extracts and all compounds exhibited antibacterial properties with respect to the zone of inhibition against *Staphylococcus aureus*, *Pseudomonas aureginous*, *Escherchia coli* and *Salmonella thyphimurium*. Compound **LC-4** (di-2-ethylhexyl phthalate) was found to be more active than other compounds and even better than the standards gentamicine in most of the tested bacteria. Maximum activity was observed against *Pseudomonas aureginous* and minimum activity was noted for *Escherchia coli.* These results also provide scientific validity and credence to the ethno medicinal use of this plant in the treatment of ailments caused by some of the pathogenic bacteria used in this study and highlights the usefulness of *Lantana camara* fruits in the treatment of bacterial infections. The observed result also confirmed that the isolated compounds are promising candidates for further antibacterial activity tests in antibacterial drug discovery. In addition, the compounds isolated are not the only compounds present in the *Lantana camara* fruits extracts as evidenced from the TLC analysis. Further work should therefore be carried out to isolate other compounds which may be more bioactive. Thus, further test is recommended on large number of bacterial strains to decide their potential as candidates in development of antibacterial drugs. And also more research should be carried out on both the crude extracts and the isolated compounds to include other antibacterial, antifungal, anti malarial and insecticidal activities.

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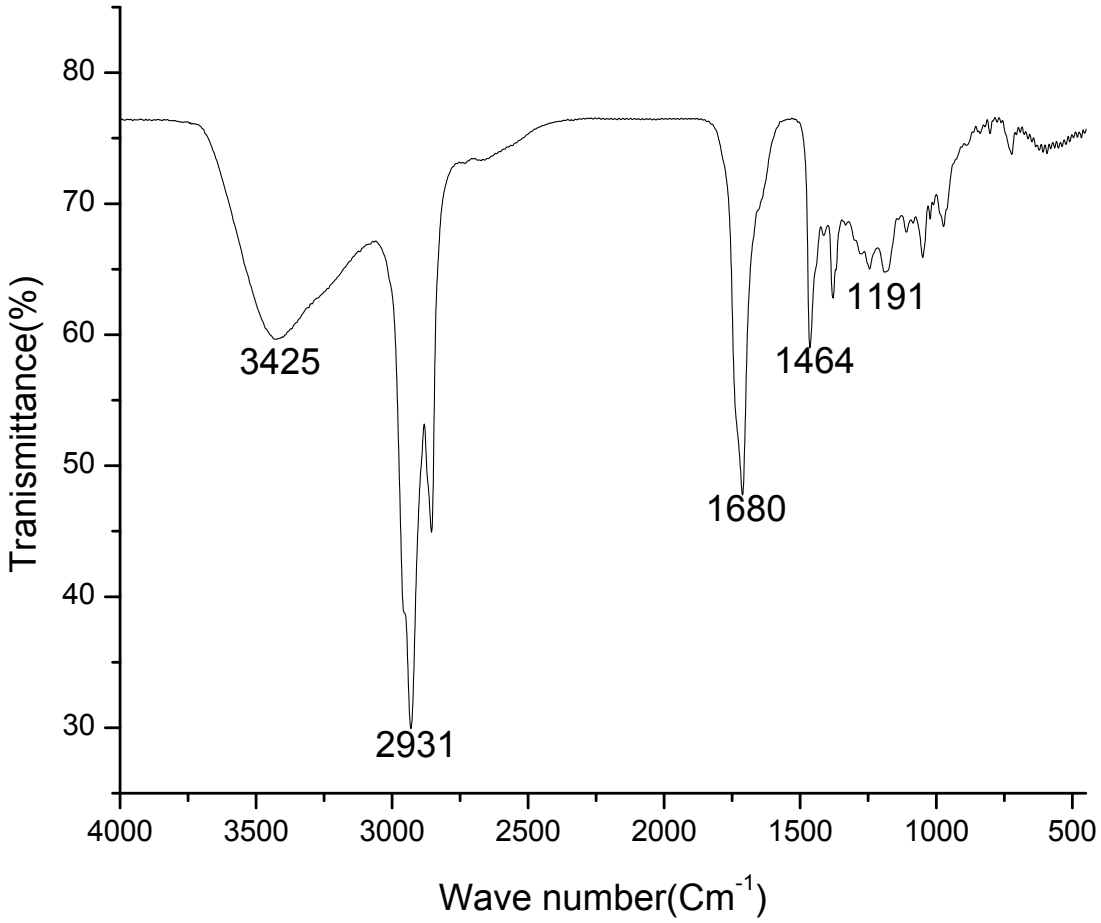
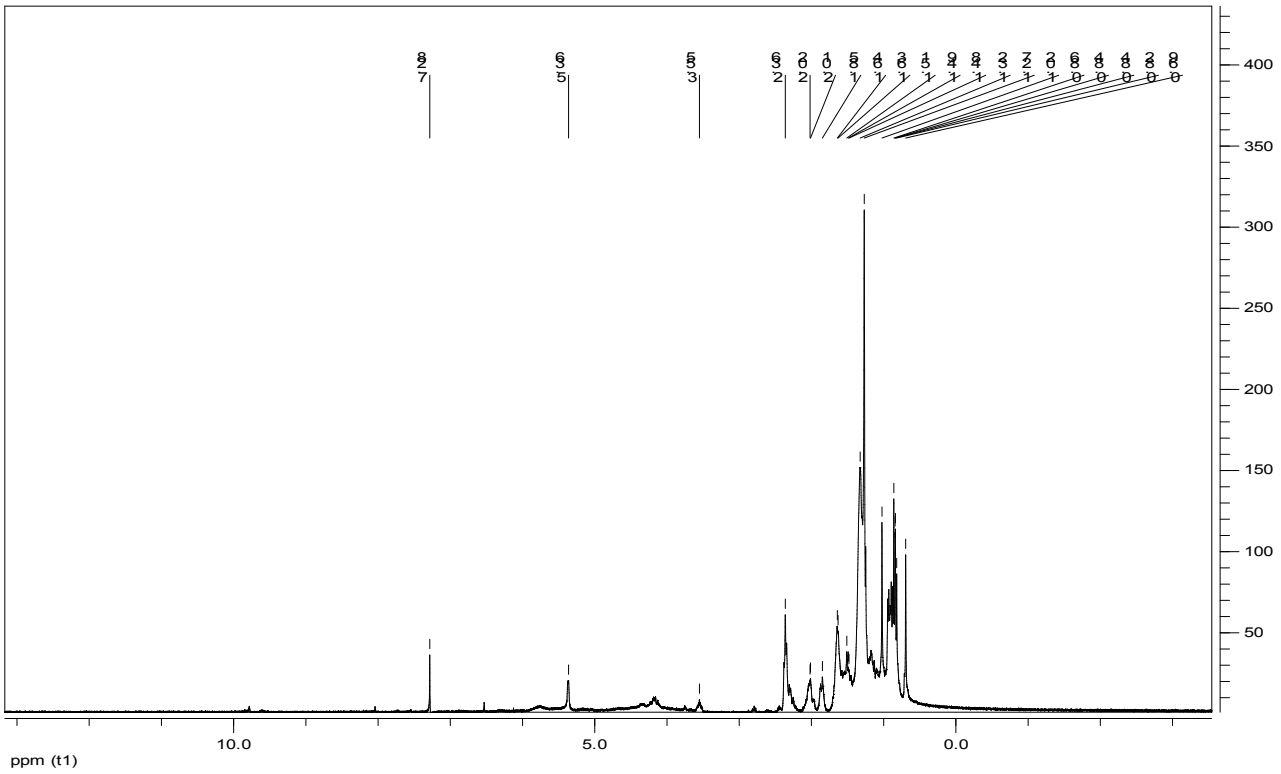
Appendices

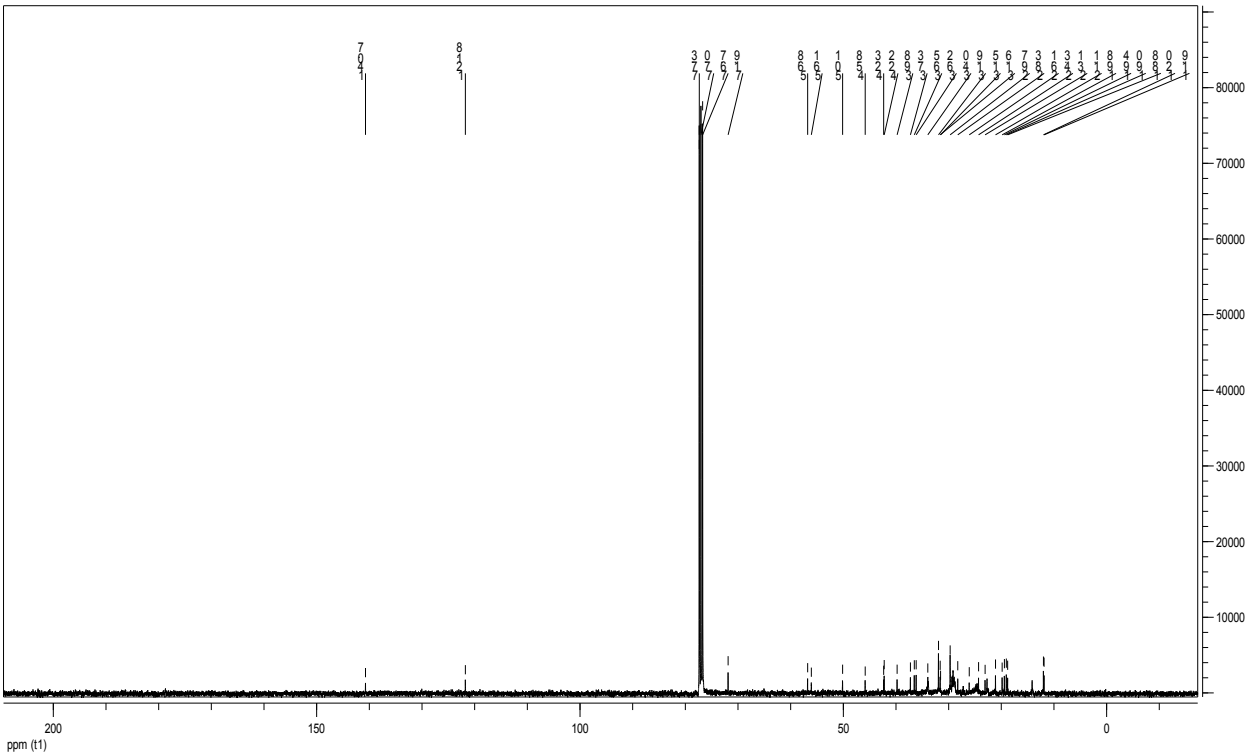
Appendix 1. IR spectrum of **LC-1**



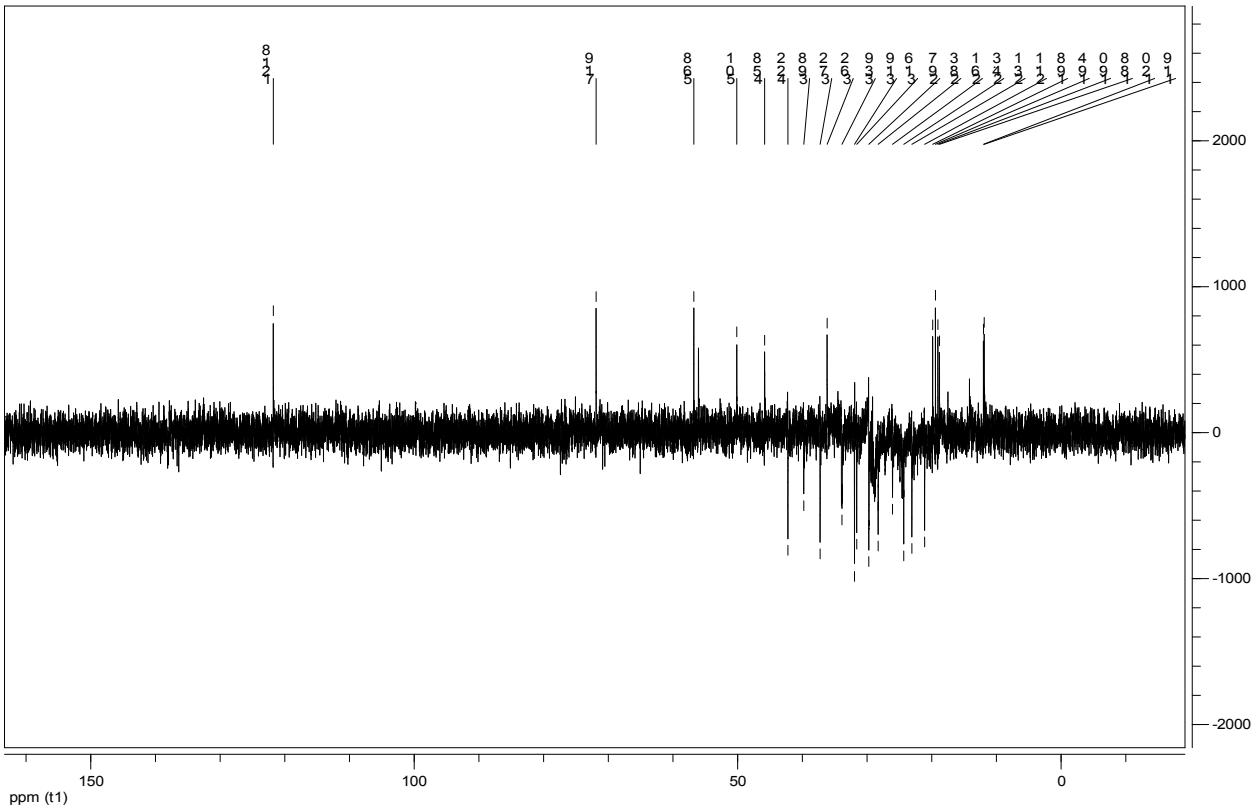
Appendix 2. ^{1}H-NMR spectrum of **LC-1** in $CDCl_3$



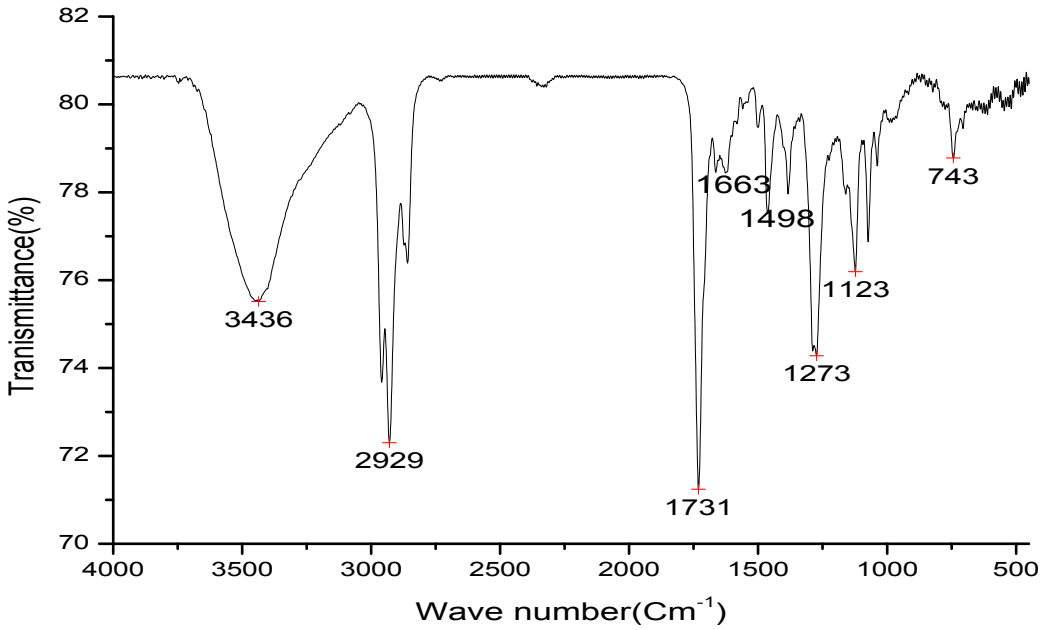
Appendix 3. ^{13}C-NMR spectrum of **LC-1** in $CDCl_3$



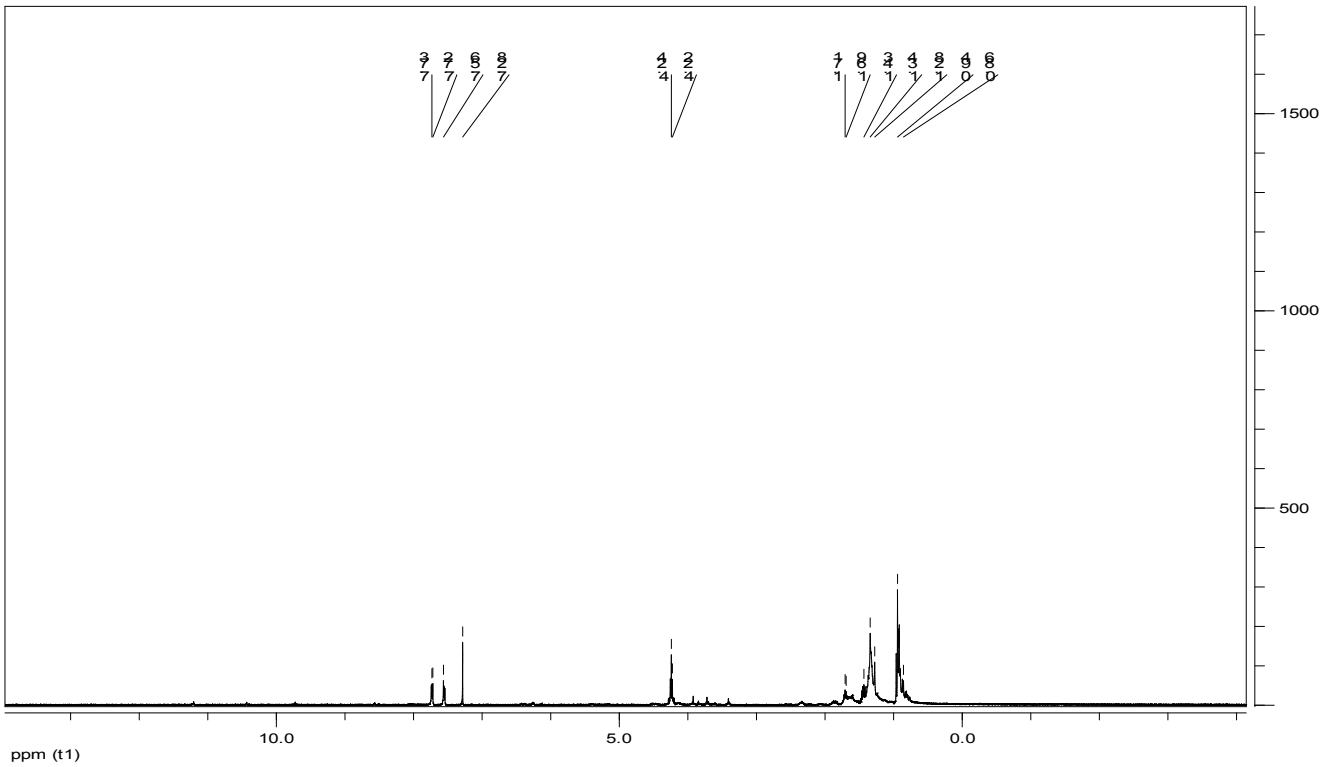
Appendix 4. DEPT-135 of **LC-1** in $CDCl_3$



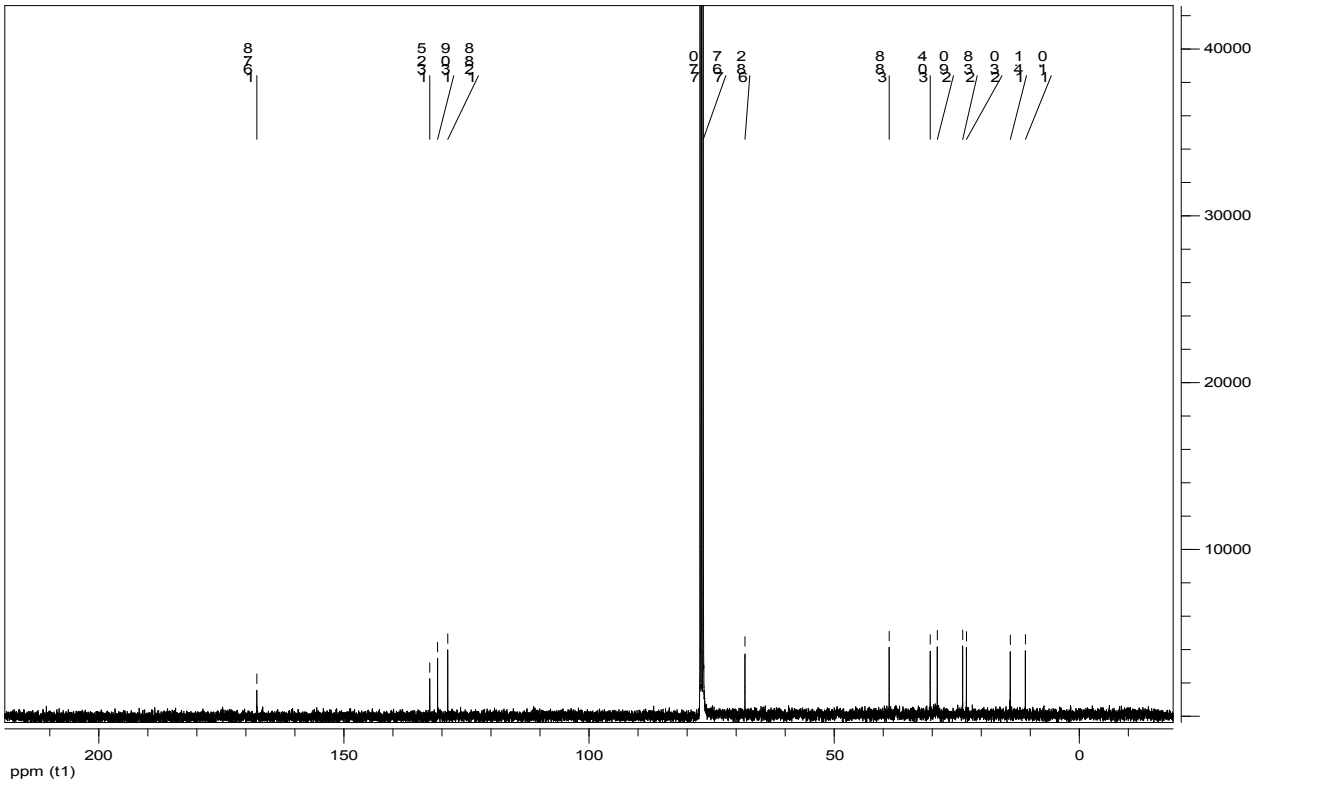
Appendix 5. IR spectrum of **LC-4**



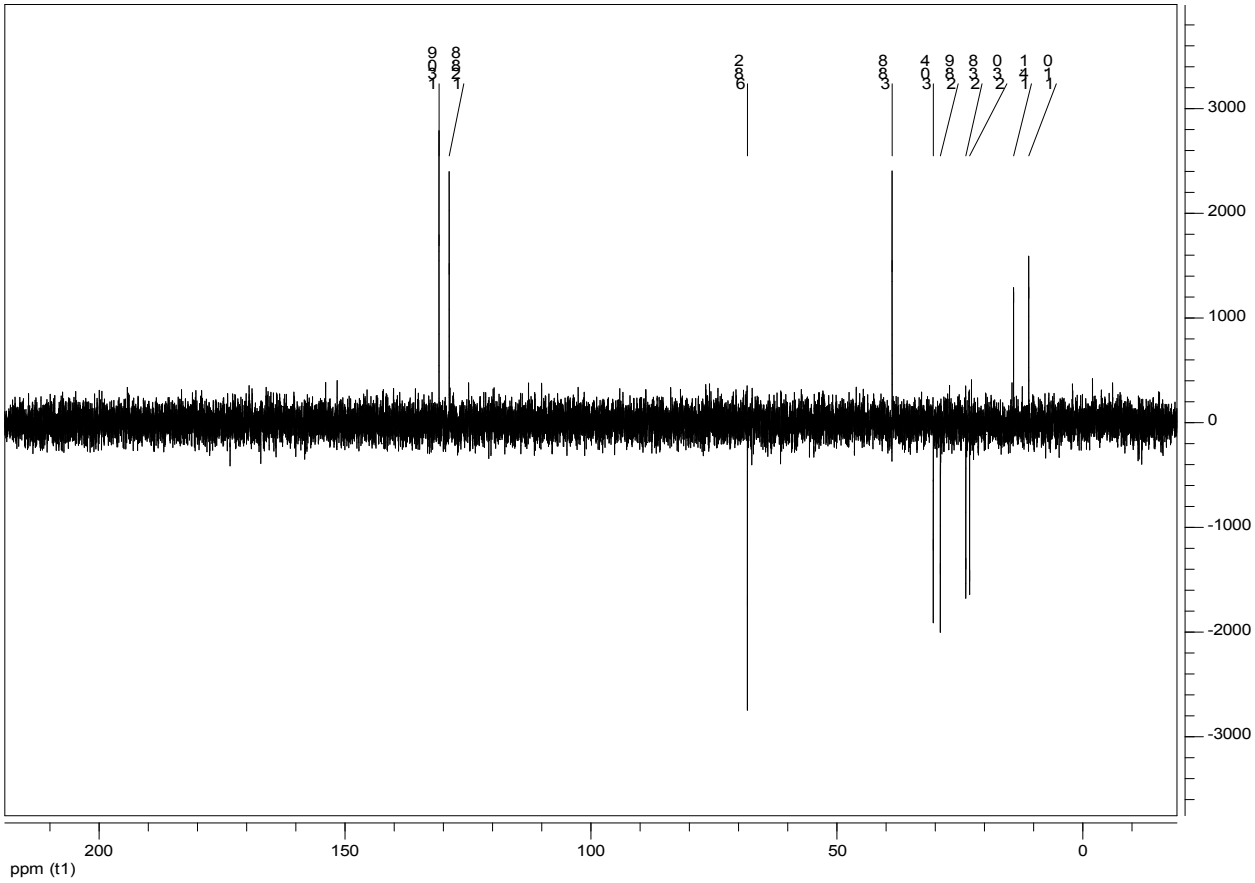
Appendix 6. ^{1}H-NMR spectrum of **LC-4** in $CDCl_3$



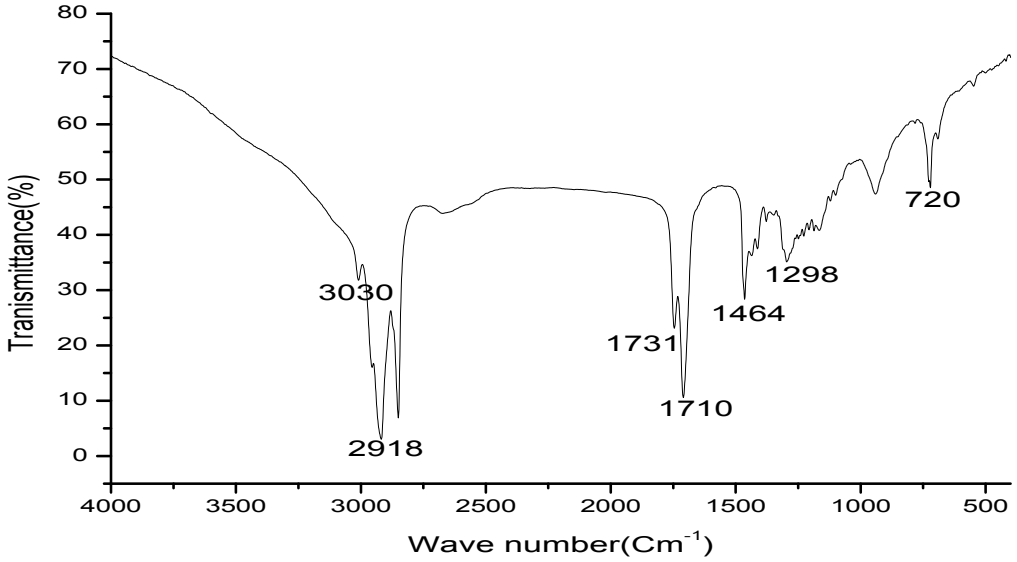
Appendix 7. ^{13}C-NMR spectrum of **LC-4** in $CDCl_3$



Appendix 8. DEPT-135 of **LC-4** in $CDCl_3$

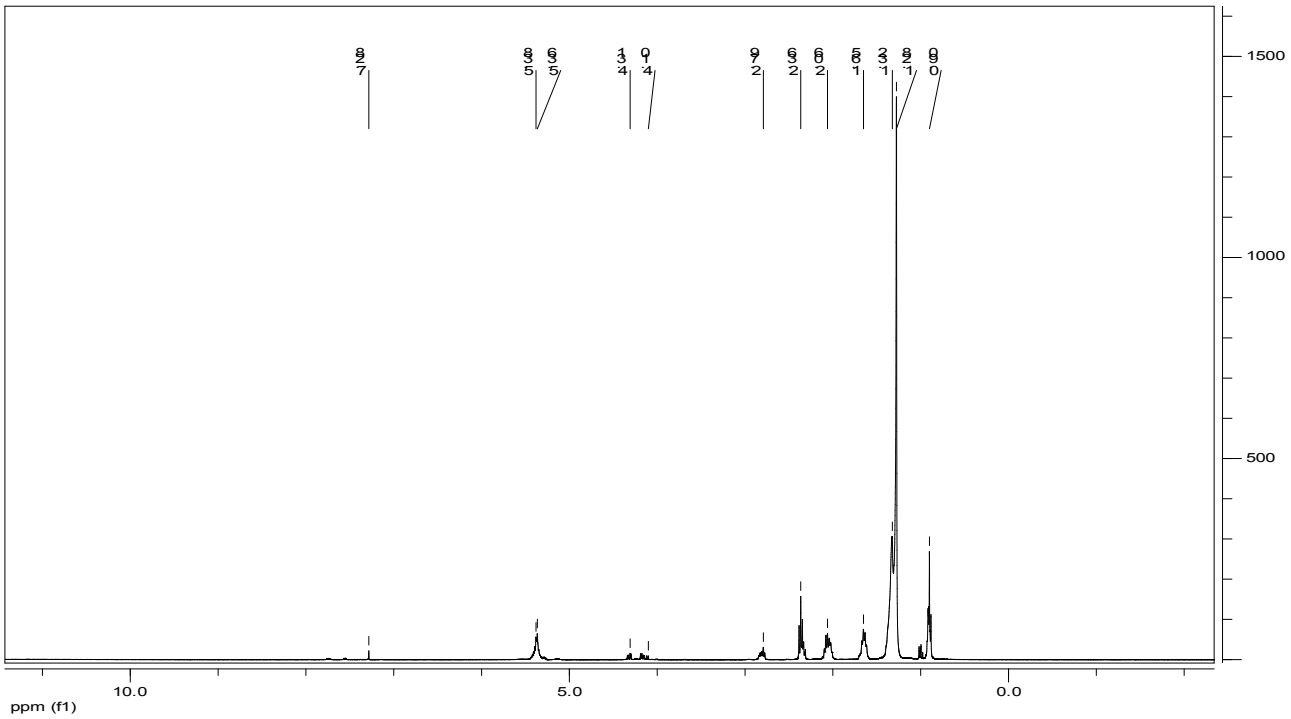


Appendix 9. IR spectrum of **LC-5** in $CDCl_3$

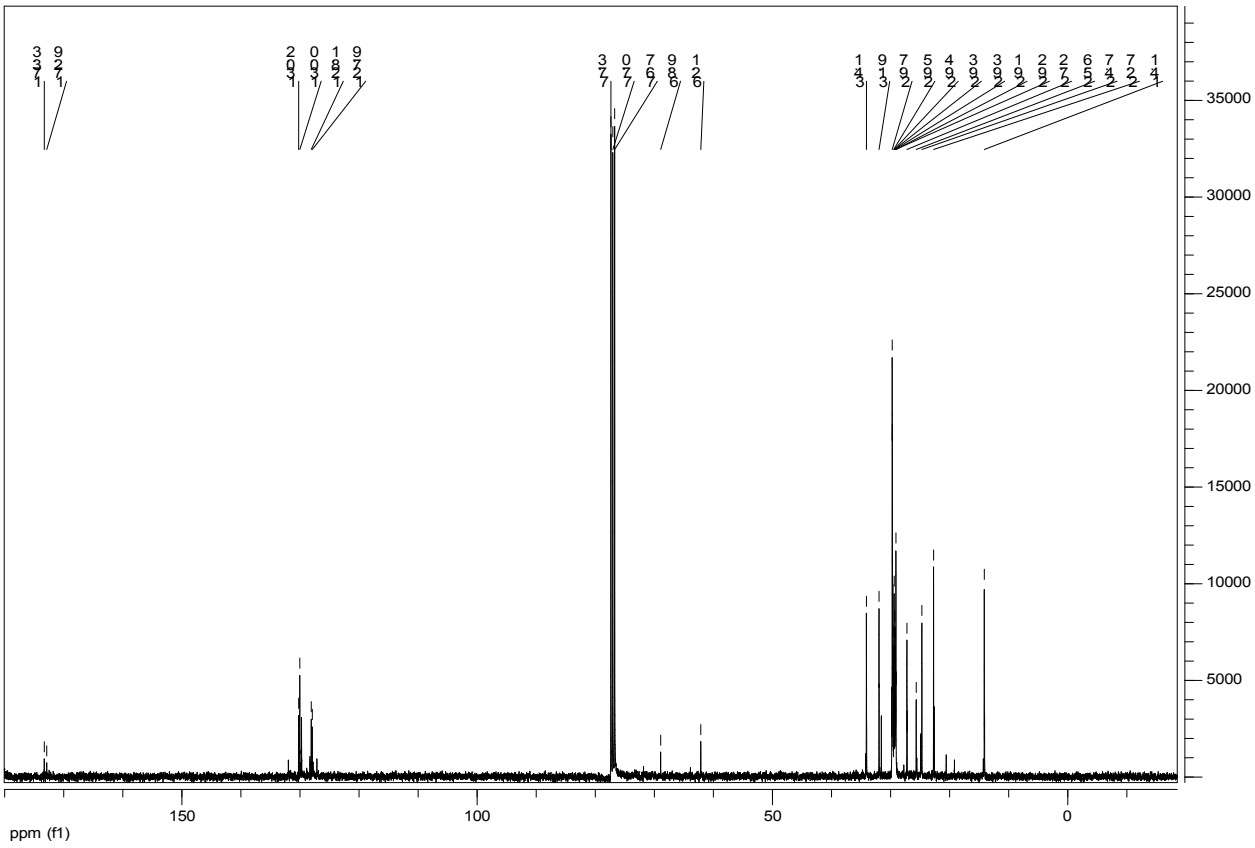


:

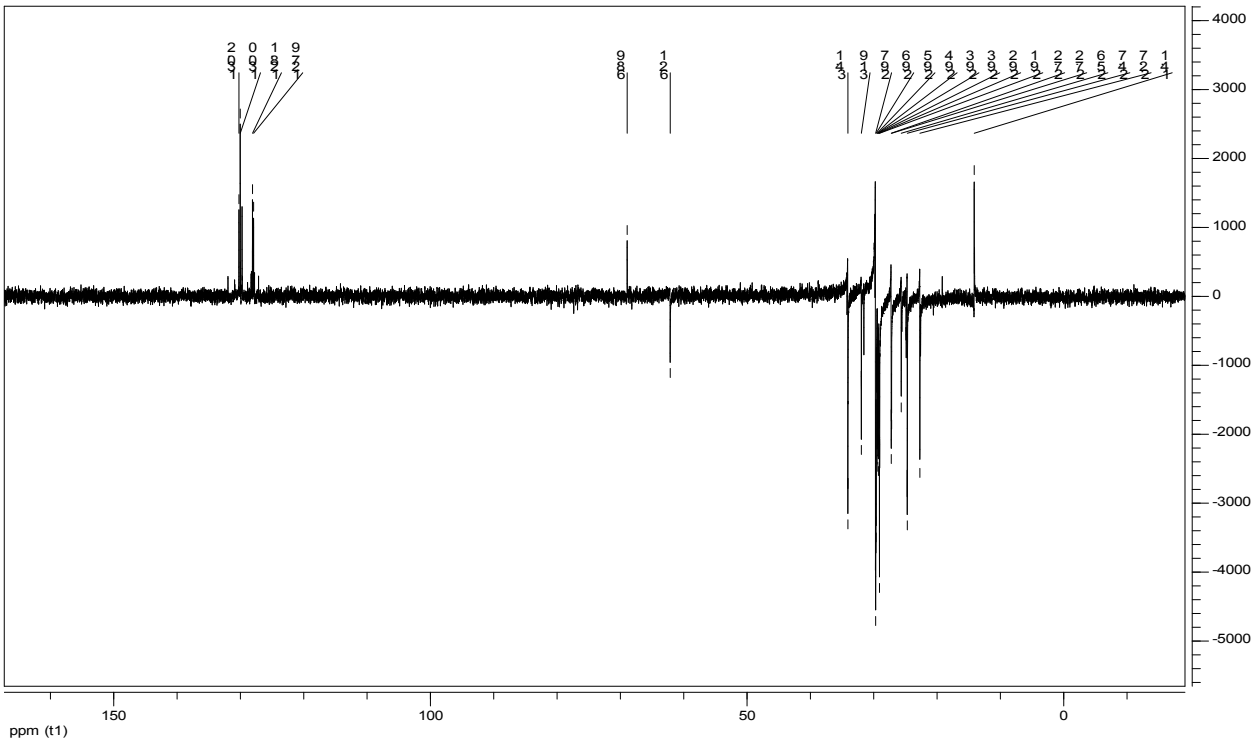
Appendix 10. ^{1}H-NMR spectrum of **LC-5** in $CDCl_3$



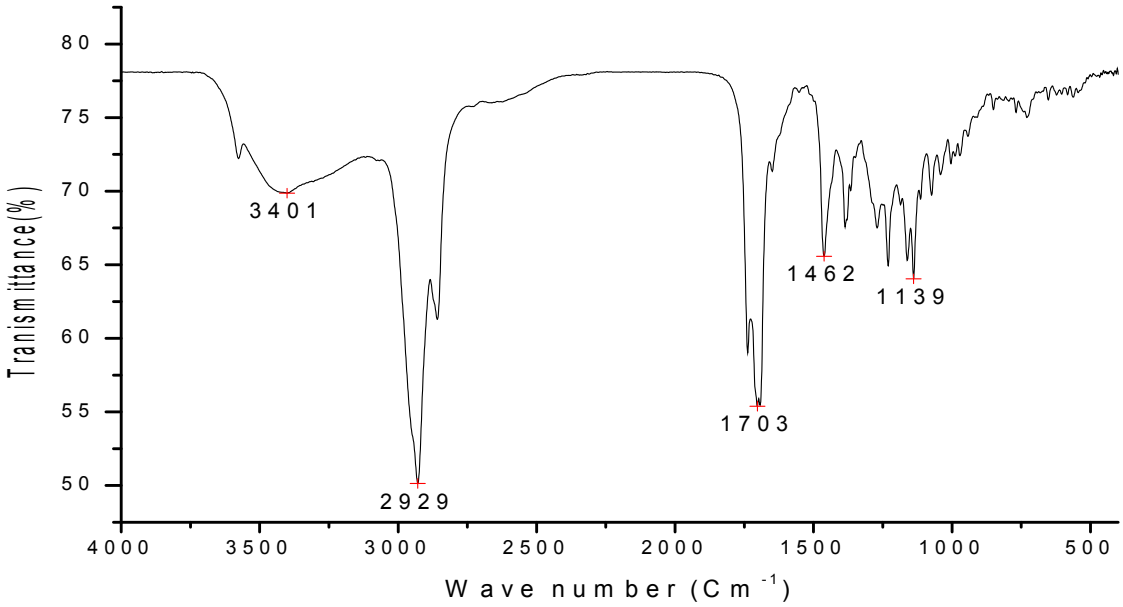
Appendix 11. ^{13}C-NMR spectrum of **LC-5** in $CDCl_3$



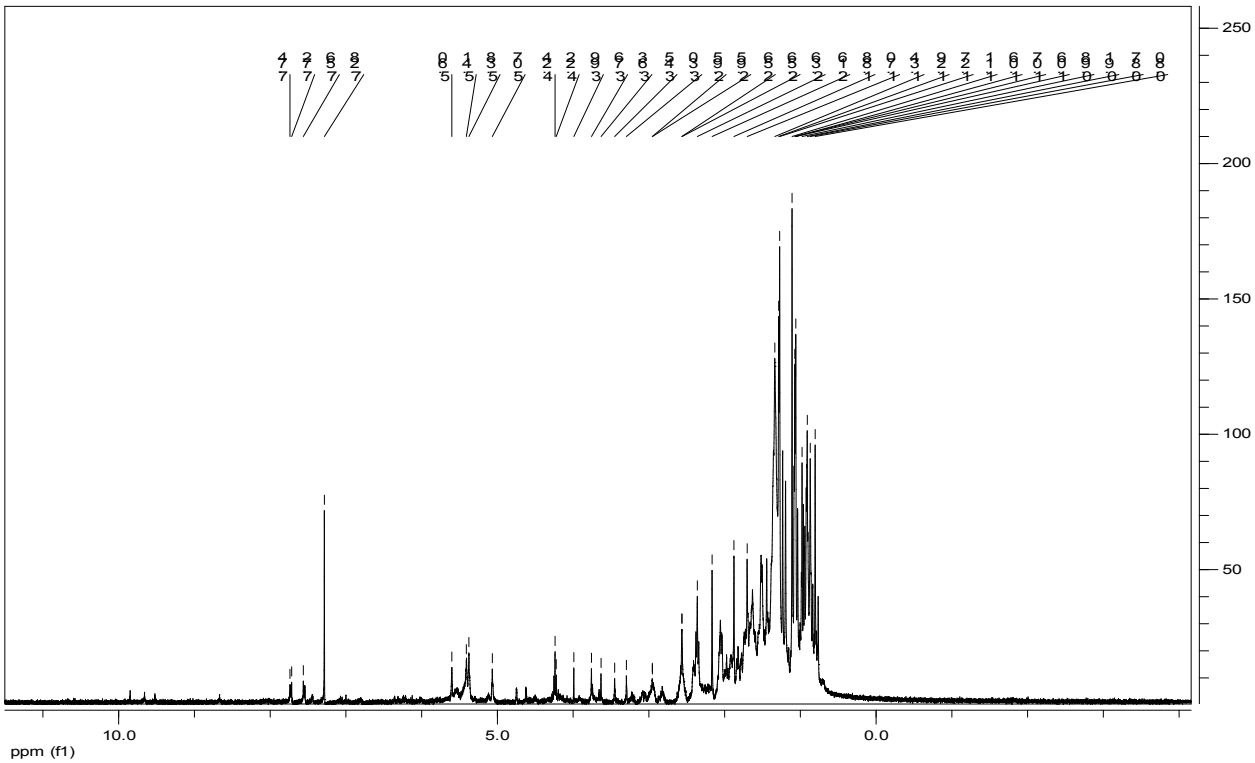
Appendix 12. DEPT-135 of **LC-5** in $CDCl_3$



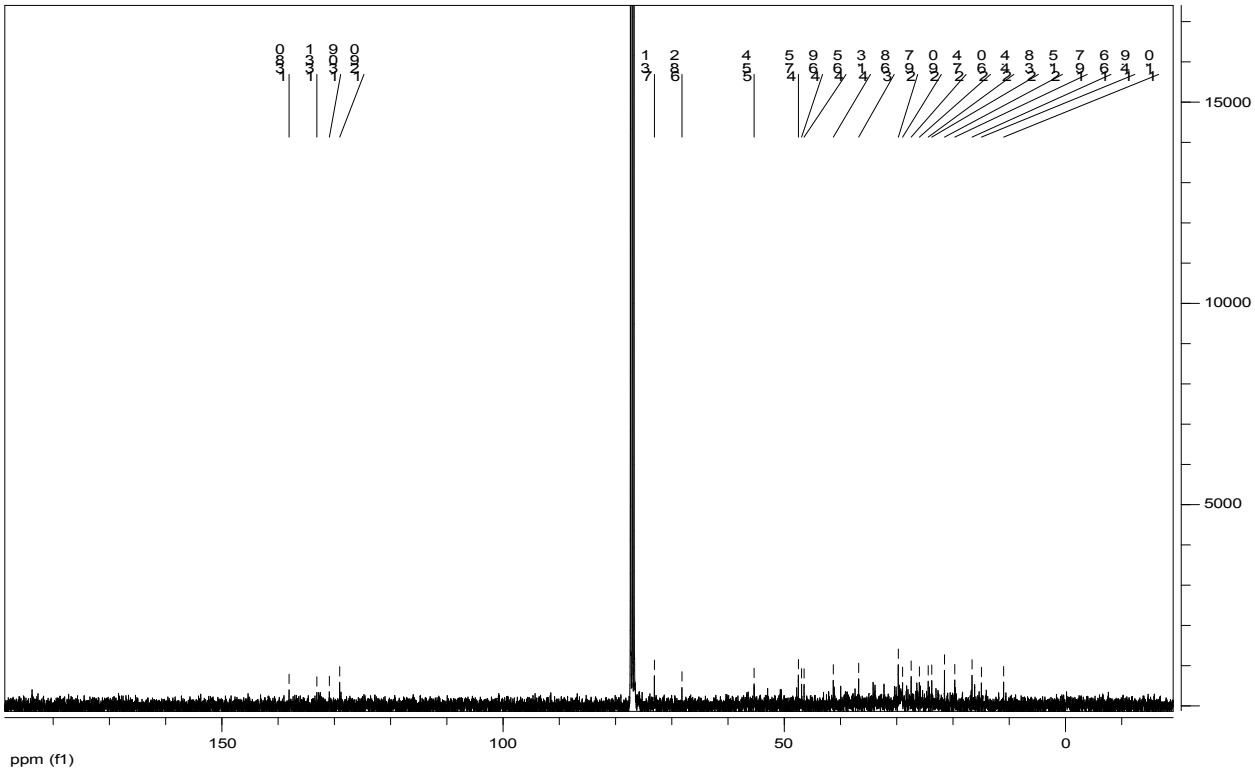
Appendix 13. IR spectrum of **LC-2**



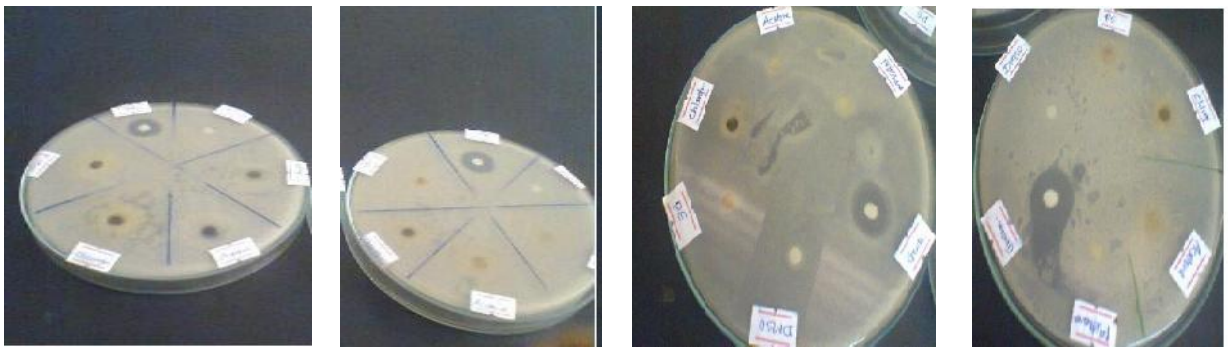
Appendix 14. ^{1}H-NMR spectrum of **LC-2** in $CDCl_3$



Appendix 15. ^{13}C-NMR spectrum of **LC-2** in $CDCl_3$



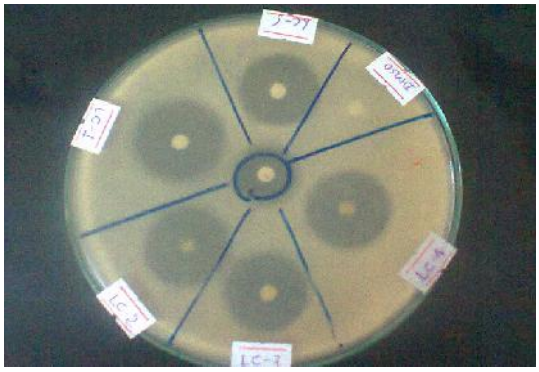
Appendix 17. Evaluation of antibacterial activity of crude extracts



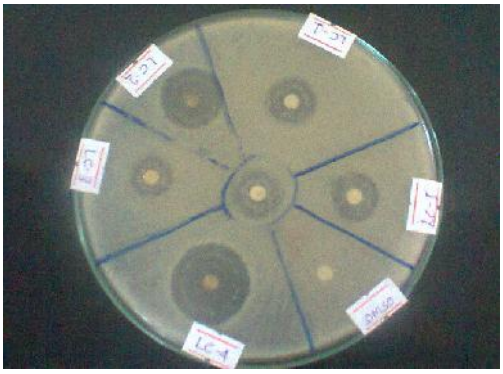
A B C D

Antibacterial activities (100mg/ml) of petroleum ether, chloroform, acetone and methanol extracts of *Lantana camara* fruit against the tested pathogens; A) *Pseudomonas aeruginosa* B) *Salmonella thyphimurium* C) *Staphylococcus aureus* D) *Escherichia col*.

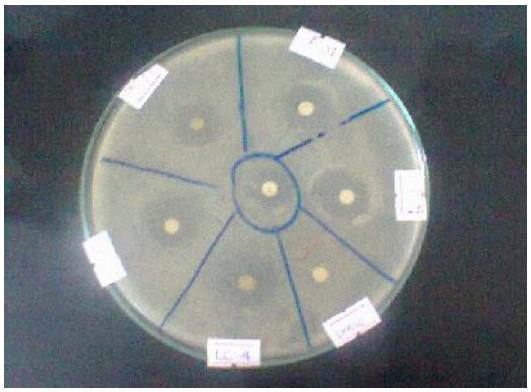
Appendix 18. Evaluation of antibacterial activity of isolated compounds



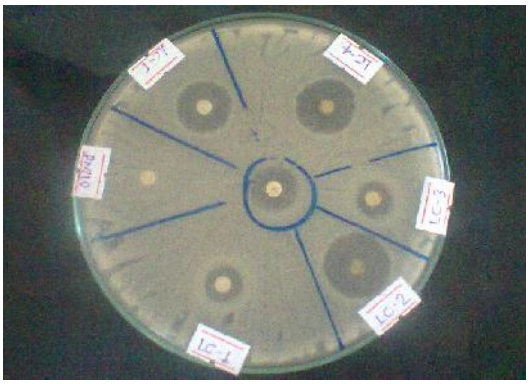
Pseudomonas aeruginosa



Staphylococcus aureus



Salmonella thyphimurium



Escherichia coli