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Chemical Investigation of the Essential oil and Solvent Extracts from the whole Aerial part of *Chenopodium ambrosioides*

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Chemical Investigation of the Essential Oil and Solvent extracts from the whole aerial part Of *Chenopodium ambrosioides*

A THESIS SUBMMITED TO DEPARTMENT OF APPLIED CHEMISTRY, SCHOOL OF APPLIED SCIENCES, ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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APPROVAL SHEET

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As thesis advisor, hereby certify that I have read and evaluate this thesis prepared under my guidance by Meti Kebede entitled Chemical Investigation of the Essential Oil and solvent extracts of *Chenopodium ambrosioides.* I recommended that it be submitted as fulfilling thesis requirement.

Dr. Hailemichael Tesso (Ph.D) _____ _____

Advisor signature date

As member of the examining board of the M.Sc. thesis defense examination, we certify that we have read evaluated the thesis prepared by Meti Kebede and examined by the candidate. We recommended that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in organic chemistry.

_____ _____ _____

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_____ _____ _____

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Statement of the author

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

Name: Meti Kebede

Signature _____

Date _____

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Abstract

In this study, the whole aerial part of a medicinal plant known as Chenopodium ambrosioides, which grows in the wild and collected from around Gedo town in the west-Shoa zone, Oromia region was investigated. C. ambrosioides is an herbaceous shrub that belongs to plants of the family Chenopodiaceae, grows wild in central and South America. It has been used for centuries by native people as dietary condiments and as traditional medicine. The plant has been used as antihelmintics, as anti-inflammatory, as anti-tumoral, as healer and as anti leishmanial which is due to the presence of a compound known as ascaridole. Previous studies conducted on the essential oil of the seeds of the plant revealed presence of a number of low molecular weight terpenes. Since no study has hitherto been conducted on this medicinal plant in Ethiopia, the whole aerial part of the plant was subjected to hydrodistillation and solvent extraction followed by GC-MS analysis, TLC analysis, chromatographic separations on silica gel columns eluted by organic solvents and preparative TLC separations. Thus, three compounds could be identified from the essential oil by GC-MS analysis. These were 1-methyl-4(1-methyledene)-cyclohexane, 1-methyl-2(1-methylethyl)-benzene and 6-isopropylidene-1-methyl-bicyclo[3,1,0]hexane.In addition, a new compound, CAC-1, was isolated from the chloroform extract of the aerial parts of the plant by repeated chromatography. The structure of CAC-1 has been elucidated by extensive analysis of the data from its ^{1}H-NMR, ^{13}C-NMR, DEPT experiments. In addition structural information about the compound has been obtained from its Infrared spectrum. By interpreting the spectroscopic data CAC-1 has been identified as 1-(4-ethoxymethyl-phenyl)-2-(4-methyl-bicyclo [1,1,1]pent-2-yl)-ethanone.

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1. Introduction

1.1 Background of the study

Plants are important in man's life and fulfill his every days needs. Different plant parts like roots, stem, leaves, fruit and twigs and modified plant organs are used as food, shelter, clothing and transportation, fertilizer, flavors, fragrances, ornamental, medicine, and cosmetics, etc. throughout the ages of humans. According to some observation, some animals like chimpanzeesutilize a number of plant species for medicinal uses [1]

The discovery of plants that serve as medicine (medicinal plants) in different parts of the world is important to agriculture and medicine sectors, because it can lead to establishment of new directions towards propagation of alternative medicinal plants that offer better economic and social benefits. The ancient civilizations such as Chinese, Egyptian, Indians, Greek, and North Africans provided written evidence for the use of natural products for treatment of various diseases [2]. The world health organization (WHO) reported that 80% of the world's populations depend on traditional medicine and a major part of traditional therapies involve the use of medical plant extracts or their active constituents [3].

Medicinal plants are used traditionally throughout the world to treat many illnesses like malaria, diabetes, respiratory and urinary tract infections, cough, fever, diarrhea, abdominal pains, pneumonia, conjunctivas, oral and tooth wounds etc. and also used for birth control and psychic problems and often exhibit a wide range of biological and pharmacological activities, such as anti-bacterial and anti-fungal properties. Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to cultures, whether explicable or not used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness [4]

Species of the family *Chenopodiaceae* are widely distributed in the East Mediterranean area, where they are often used commercially as spices or drugs because of the presence of useful secondary metabolites. The most characteristic constituents are flavonols, essential oils and terpenes [5-6].

The genus *Chenopodium* includes varieties of weedy herbs (more than 200 species) native to much of Europe, Asia, India, China and both North and South America. Goose foot is common name for the genus *Chenopodium*, as well as for the goose foot family Chenopodiaceae. Various

plant parts of different species of *Chenopodium* have been traditionally used in the treatment of several disorders [7]. *Chenopodium ambrosioides* oil (also known as American wormseed oil, *Chenopodium* oil, or Baltimore oil) is rich in monoterpenes [8]

The seeds and fruits contain large amount of essential oil that has a main active compound in it called ascaridole (**1**). Herb of *C. ambrosioides* is a plant widely known in popular medicine as antihelmintics, vermifuge, emmenagogue and abortifacient [9-10]. However, to the best of my knowledge, no chemical investigation of *C ambrosioides* (Amedmado Amharic name) has been hitherto conducted in Ethiopia. Therefore, in this study the aim was to carry out the chemical investigation of the essential oil and solvent extract from the whole aerial parts of *C. ambrosioides* that was obtained from around the town of Gedo, which is located about 167 km west of Addis Ababa, western Oromia, Ethiopia.

1.2 Botanical Description

This plant (Fig. 1) has a strong aromatic odor and grows to a height of 1m. It blooms and fruits when only 4 cm tall or can reach its maximum height of 1m tall before flowering. The flowers are small and green, produced on a branched panicle at the apex of the stem. It occurs in temperate region with annual average temperature ranging from 5°-35°C and annual rain between 100-2000 mm.

Figure 1: *Chenopodium ambrosioides* plant

1.3 Significance of the study

The result of this study would:-

- Provide additional information about the chemical compositions of essential oil and solvent extracts of the whole aerial parts of *C. ambrosioides*
- Would help to identify new compound that can be used in drug discovery.

1.4 Objectives

1.4.1 General objective

- The main objective of this study was to study the chemical composition of essential oil and solvent extracts of the whole aerial parts of *C. ambrosioides*.

1.4.2 Specific objectives

- To extract the essential oil of the whole aerial part of *C. ambrosioides*.
- To analyze the chemical composition of the essential oil by GC and GC-MS
- To extract the whole aerial part of the plant by acetone, chloroform, ethyl acetate and hexane.
- To isolate compounds from chloroform solvent extracts.
- To characterize compounds isolated using different spectroscopic (NMR and IR) methods.

2. Review of Related Literature

2.1 Medicinal value of *C. ambrosioides*

C. ambrosioides belongs to family *Chenopodiaceae* and grows wild in central and South America. It is an herbaceous shrub that has been used for centuries by native people as dietary condiments and as traditional medicine. *C. ambrosioides* has been used as antihelmintics, as anti-inflammatory, as anti-tumoral, as healer and as anti leishmanial which is due to presence of a chemical compound known as ascaridole (1). Ascaridole is formed in highest concentration in the seeds. Other components found in the essential oils of the plant are limonene, *trans*-pinocarveol, ascaridol eglycol, aritasone, α-pinene, myrcene, phellandrene and α-terpineol. Some uses of *C. ambrosioides* include expulsion of intestinal worms and parasites, against skin parasites, for lice and ring worms, to balance and strength the liver (for liver flukes and parasites) balance and strength the stomach and bower (for acid reflux intestinal gas, cramping, chronic constipation, hemorrhoids, etc) for coughs, asthma, bronchitis and other upper respiratory problems [11]. The plant is reported to be used as analgesic (pain reliever), antacid, anti inflammatory, anti-hepatoxic (liver detoxifie, antimicrobial, antiseptic, antispasmodic, antiulcer, carminative, contraceptive, diaphoretic promotes uleating) digestive stimulant, gas trototonic, (tones, balance, strengthens) hepatoprotective (liver protector), laxative, lactagogue (promotes milk flow) menstrual stimulant, newrvine (balance/calms nerves) sedative, tonic (tones, balances, strengthens overall body function, wound healer[11].

2.2 Some compounds isolated from genus of *Chenopodium*

Some compounds whose structures are given in Figure 2, such as ascaridole (1), terpinene (2), p-cymene (3), thymol (4), limonene (5) and camphor (6) have been reported from various species of the genus *Chenopodium* [12].

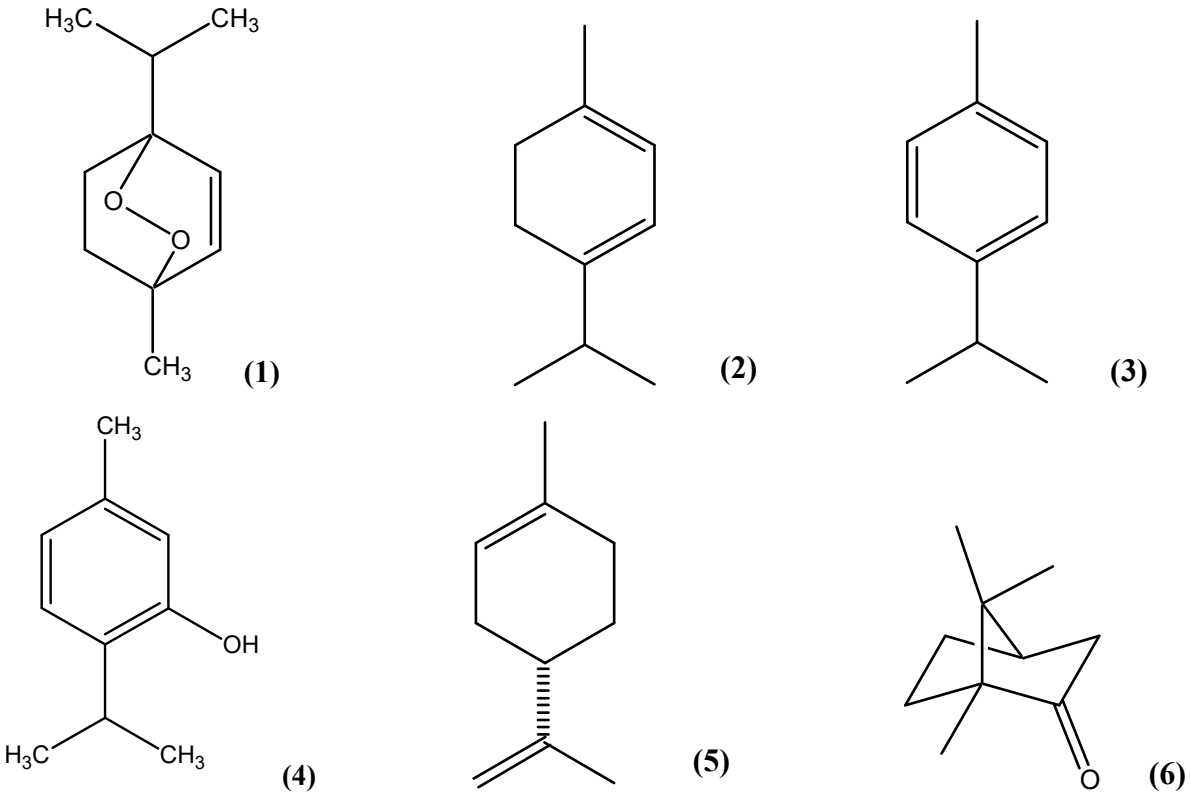


Figure 2: The chemical structures of compound (1-6) isolated from genus of chenopodium.

2.3 Screening of plant extracts for various phytochemical

The following phytochemical tests were performed for observed different chemical groups present in crude extracted.

2.3.1 Alkaloids

Alkaloids are basic substances that contain one or more nitrogen atoms, usually in combination or as part of cyclic system. They have basic properties and are alkaline in the reaction turning red litmus paper blue due to the presence of one or more nitrogen atom that are in an alkaloid, usually 1^0, 2^o or 3^o amine, contribute to the basicity of alkaloid. Most of alkaloids exist as solid such as atropine, some as liquids containing carbon, hydrogen and nitrogen, moreover, the alkaloids are readily soluble in alcohol and sparingly soluble in water but their salts are usually soluble. Alkaloids are used in the defense of plant against herbivores and pathogens and are widely exploited as pharmaceuticals, stimulants, narcotics and poisons due to their potent biological activities. In nature, alkaloid exists in large proportions in seeds. Based on their

structures, alkaloid can be subdivided as heterocyclic and cyclic alkaloids [13]. Some of the compounds are listed as follow. Quindine alkaloide (7), Indole (8), Theophylcine (9), Tropane (10) (figure :3)

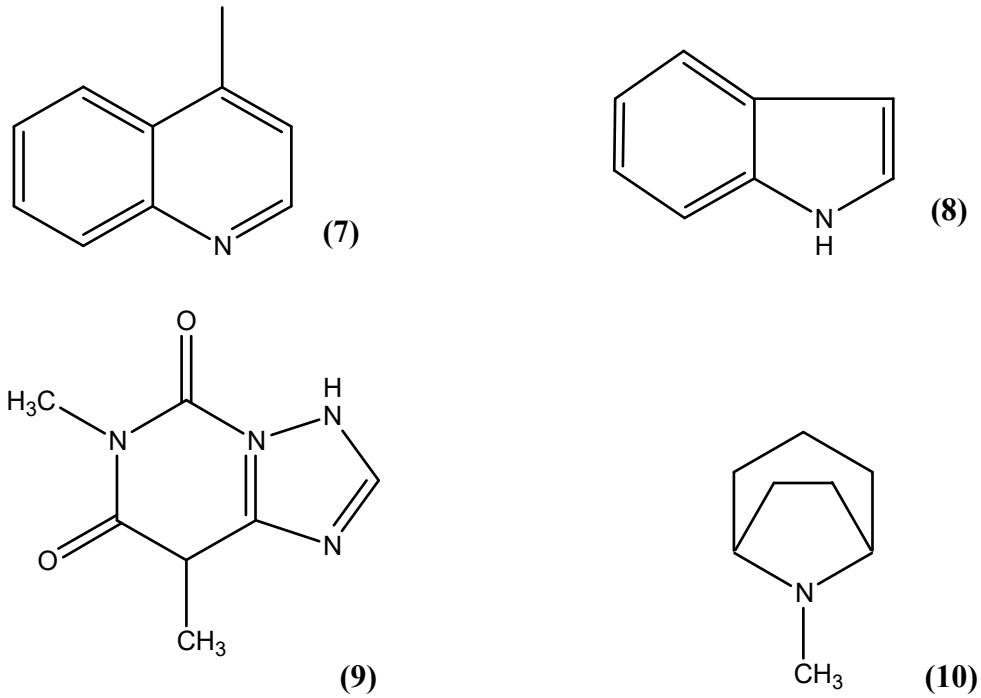


Figure 3: The chemical structure of some alkaloids (7-10)

2.3.2 Phenols

There are secondary metabolites characterized by the presence of one or more hydroxyl (OH) bonded to aromatic ring. Phenols or phenolic or polyphenolics are chemical components that occur as natural color universally pigments responsible for the color of fruits of plants. The most important role in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections.

Phenolic are found in apples, green-red-tea and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents[14]. Examples: Ferulic acid (11), Caffec acid (12)(figure :4)

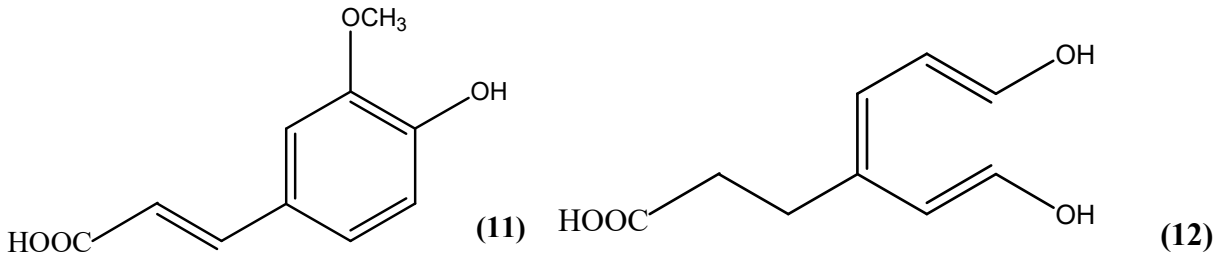


Figure .4: The chemical structures of some phenolic compounds (**11-12**)

2.3.3 Flavonoids

Flavonoids are secondary metabolites that are large and most diverse group (poly) phenolic compounds distributed in plant kingdom accumulating in different part like bark, leaves, flowers, root, stem fruits and seed. Flavonoids are structurally characterized by the flavones nucleus which consists of 15 carbon atom arranged in three rings [15]. Some of the flavones extracted from the plants are: Flavonenes (13), Isoflavonoid (14), Flavonis (15) and Isoflavonoid (16)(figure:5)

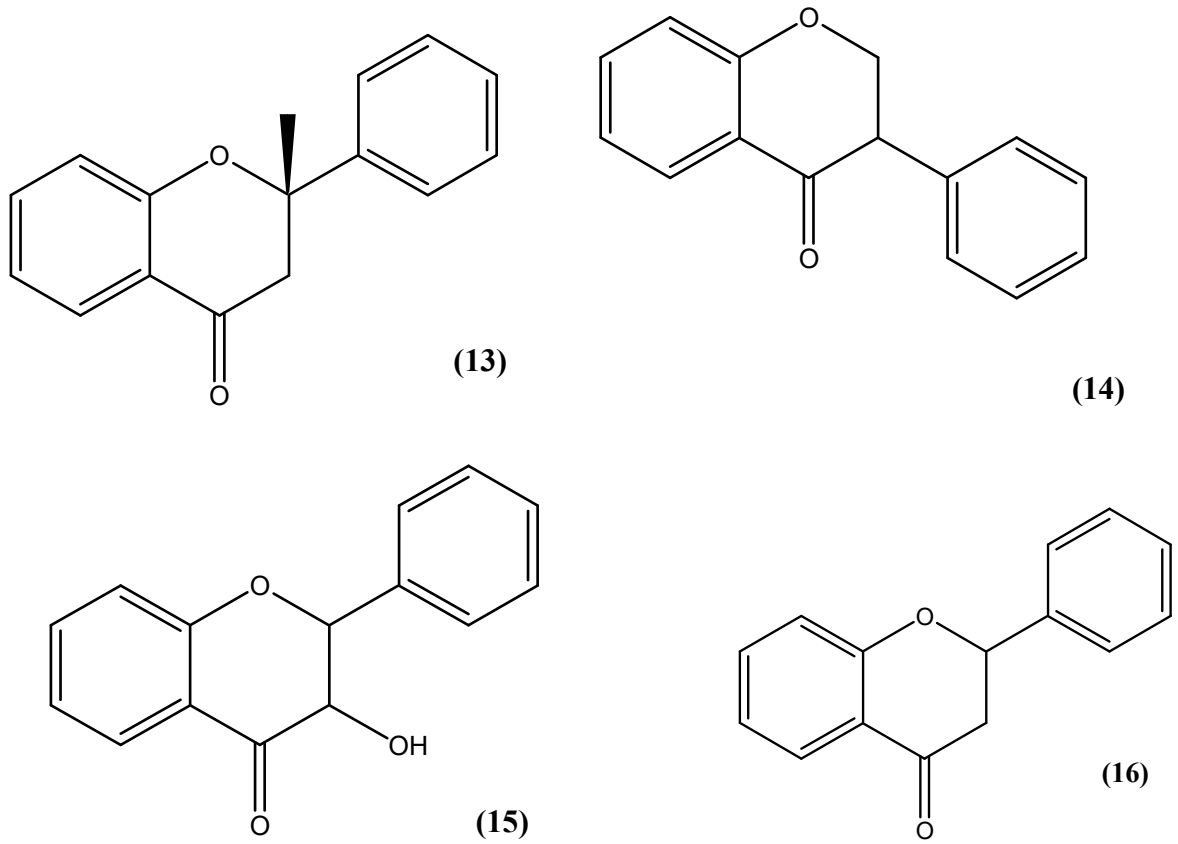


Figure 5: The chemical structure of some compound of flavonoids (**13-16**)

2.3.4 Tannins

Tannins are naturally occurring poly phenolic compounds with high molecular weights which are characterize by precipitating (due to multiple OH groups) biological molecules like proteins, alkaloid and distributed in plant flora. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layer of plant tissue [16] .Tannins rich medicinal plants are used as treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea [16]. Examples: Ellagic acid (17), Gallic acid (18)(figure:6)

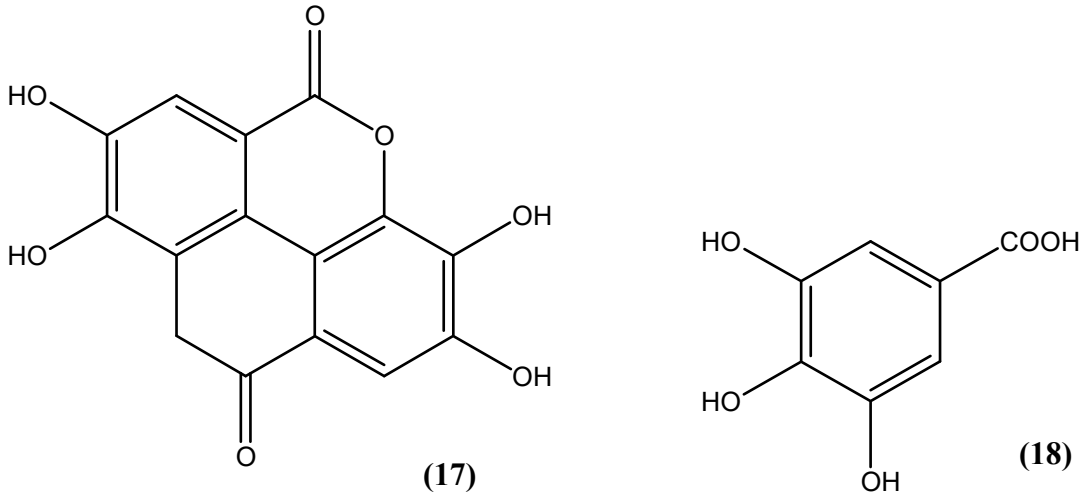


Figure 6**:** The chemical structures of some tannin compound (**17-18**)

2.3.5 Saponins

Sapanaria vaccaria rich in saponins which was once used as soap and they are natural detergent found in many plants, especially certain desert plants and therefore possess" soap like" behavior in water (produce foam)[17]. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvent like benzene and n-hexane. Saponins are important therapeutically as the show to have hypolipidemic and anticancer activity [17]. Some Saponins extracted are: Quillaja acid (19) and Hederagenins (20).(figure:7)

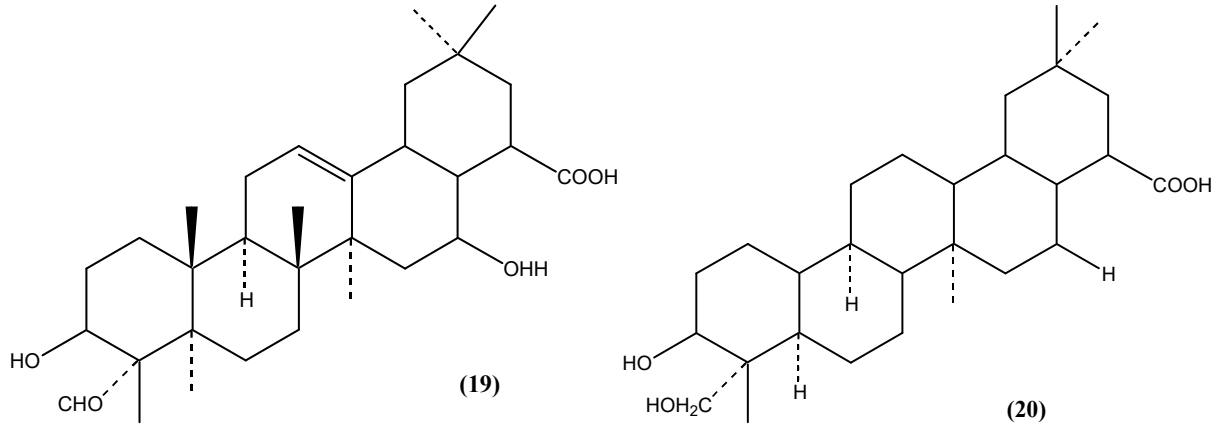
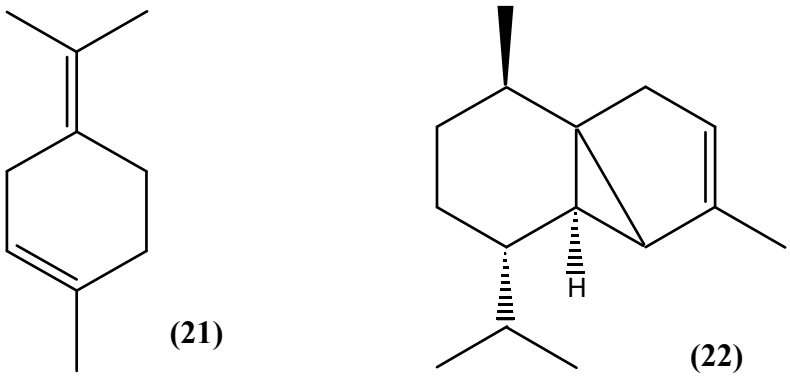


Figure 7**:** The chemical Structures of some Saponins compound (**19-20**)

2.3.6 Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins. Terpenoid includes hydrocarbons of plant origin of general formula (C_5H_8) n and are classified as mono, di, tri-and sesquiterpenoids depending on the number of carbon atoms, Examples of commonly important monoterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes are classically considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, Insecticidal or cytotoxic activity. Common triterpenes: amyrins, ursolic acid sesquiterpene like monoterpenes, are major components of many essential oils [18].The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxin action. The sesquiterpene lactones, palasonin, isolated from Butea monospermous has antihelmintics activity, in habits glucose up take and depletes the glycogen content in Ascaridia galli. Terpenoid are classified according to the number of isoprene units involved in the formation of these compounds. Some of the compounds are: terpenolen (21) and cubebene (22). (figure:8)



(21) **(22)**

Figure 8: The chemical structures of some terpenes

2. 4 Extraction techniques

2.4.1 Solvent extraction

Solvent extraction is usually used recover a component either solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Some extraction techniques involve partition between two immiscible liquids: others involve either continuous extractions or batch extraction.

2.4.2 Hydrodistillation

This was the main method used for extraction of the analyzed essential oils. The process is carried out in a Clevenger type apparatus, where the material to be extracted is chopped, and immersed in water, which is then boiled. During hydro distillation the essential oil components form a mixture with water. The vapors of the volatile components are carried by the steam to a condenser. The distillation period can take from 2 to 2.5 hr. The extraction period influences not only the yield but also the extract composition.

2.5 Chromatographic Techniques

Chromatogram is separation techniques in which the analyst in the mixture are separated based on their interaction with both mobile and stationary phases. Separation of the analyst is depending on the difference in physical property like molecular size, solubility and so on. Some of chromatographic techniques use in this work is discussed below.

2.5.1 Gas chromatography (GC)

GC is the of a carrier gas to convey the sample in a vapor state through narrow column made from usually fused silica tubes (1.0 to 0.3mm ID) that have refined stationary phase films (1.0 to 5cm) bound to the surface and cross linked to increase thermal stability .The column is installed in an oven that has temperature control, and the column can be slowly heated up to 350_450c starting from ambient temperature to provide separation of wide range of compound. The carrier gas is usually hydrogen or helium under pressure, and the eluting compound can be detected several ways, including flames flame ionization detector (FID) by changes in properties of the carrier (thermal conductivity detector), or mass spectrometry(MS).The availability of ``universal" detector such as the FID and MS, makes GC the appropriate tool in the investigation of essential oils. However, GC is restricted to molecules (or derivatives) that are sufficiently stable and volatile to pass through the GC system enact at operating temperatures [19, 20, 21].

2.5.2 Gas chromatography/Mass spectrometry (GC/MS)

In GC/MS a mixture of compounds to be analyzed is initially injected into the GC where the mixture is vaporized in a heated chamber (injector).the gas mixture travels through GC column carried by a gas, where the compounds became separated as they interact with the stationary phase of the column .the separated compound the immediately enter the mass spectrometer that generates the mass spectrum of the individual compounds.

2.5.3 Thin layer chromatography (TLC)

Chromatography is used for separation, isolation, identification and quantification of component in a mixture are carried through the stationary phase by the flow of a mobile phase (solvent).Separations are based on differences in migration rates among the sample components. It is a simple, quick and in expensive procedure that can be used for the analysis of mixtures [22]. In TLC the sample is applied as a small spot or streak to the origin of a thin layer such as silica gel, aluminum, cellulose powder, polyamides, ion exchangers or chemically the elute or mobile phase is a mixture of organic and/or aqueous solvent in which the spotted plate is placed. The mobile phase moves through stationary phase by capillary action, sometimes assisted by the gravity or pressure [23, 24] Plates can be visualized depending on the chemical structure of the compound at visible light UV lamp at 365 nm or by using spray reagent [25] the effectiveness of the separation depends on mixture to be separated, choice of the mobile phase and adsorption layer. The RF value is the distance sample moved divided by distance the solvent front has

moved. RF =the distance the sample moved/the distance the solvent front moved. Usually the center of each spot is the point taken for measurement. Competition of Rf values makes it possible to research complex mixtures qualitatively and number of components in mixture is easily predicated [26].

2.5.4 Column chromatography (CC)

This method is purification technique to isolate unknown compounds from a mixture in which the stationary phase is placed in a vertical glass column and the mobile phase, a solvent, is added to the top and flows down through the column (by either gravity or external pressure). Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different constituents in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components or elute are collected as the solvent drips from the bottom of the column. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvent the polar molecules rapidly through the column. As the solvent become more polar the movement is very rapid and the separation become poor or no separation will takes place.

2.5.5 Preparative TLC (PTLC)

PTLC has been a popular method as a primary or final purification step in an isolation procedure. The adsorbent thickness of PTLC is 0.5-4mm whereas that of analytical TLC is o.1-2.mm.For commercially available PTLC plates; adsorbents (silica, alumina, C18, and cellulose) are usually 0.5, 1.0 and 2.0mm in thickness [27].

2.6 Structure elucidation

Structural elucidation is total determination of the structure of unknown compound depending on the information from literature, physical property of the compound and chemical method. Additionally different spectroscopic techniques like UV-visible, infrared (IR), nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) are used in elucidating the structure of unknown compound.

2.6.1 Nuclear magnetic resonance (NMR) spectroscopy

NMR is the most widely used spectroscopic technique giving detailed information in elucidating the structure of simple and complex compounds isolated from natural product. It involves the absorption of radio frequency radiation by atomic nuclei in an applied magnetic field. Any atomic nucleus which possessed either odd atomic or odd atomic number or both has angular momentum and a magnetic moment. ^{1}H, ^{13}H, $^{19}F.^{31}P$, ^{15}N, have nuclear spin [28-29].

^{1}H NMR and ^{13}C NMR are used in elucidating structure of compounds. ^{1}HNMR provides information about the number of protons present in the molecule, the position of the signals (the chemical shift) reveals information regarding the chemical and electronic environment of the protons, and the splitting pattern provides information about the number of neighboring(vicinal or germinal) protons[29]. The abscissa shows the chemical shift t (ð) values of the different type of protons and the ordinate shows the intensities of the signals. The signals of protons of the chemical shift value in the ^{1}HNMR is normally 0-12 ppm. The signals of protons attached to the saturated atoms like methyl, methylene and methane groups appear between 0.8 and 2.4 ppm. The most intense peaks arise from the methyl groups. The less intense peaks arise from both the methylene as well as the methane groups. The signals between 4.25 and 4.35 ppm correspond to the olefinic methane groups. Furthermore, the signal from 7.5 5-7.75 ppm is typical for ketone groups [29].

Similar to ^{1}HNMR, NMR is a plot of signal arising from the different ^{13}CNMR signal as a function of chemical shift. The signals appear as singlets because of the decoupling of the attached protons. In ^{13}CNMR different types of carbons such as primary, secondary, tertiary and quaternary carbons are identified. The range of the chemical shift values in ^{13}CNMR is normally 0-230 ppm, and this wide range allows the peaks to be more widespread making structure elucidation easier. The most important future of ^{13}C-NMR for determining multiplicities of carbon atoms is distort on less Enhancement by polarization Transfer (DEPT). This technique determines the number of hydrogen attached to a given carbon. In DEPT-135, methine and methyl carbon give positive peaks, whereas methylene carbons appear as inverse peaks [28]

3. Materials and Methods

3.1 Plant material

The aerial part of *C. ambrosioides* was collected from its natural habitat in Gedo town, Challia woreda, Oromia regional state, during the period of September-November, 2014. The plant was identified by botanists from Addis Ababa University and a voucher specimen has been deposited in the National Herbarium, Addis Ababa University.

3.2 Apparatus and Instruments used

Polyethylene plastic bags, ceramic mortar and pestle, grinder, beakers, reagent bottle, digital analytical balance, round bottom flask, Clevenger apparatus and heating mantel, TLC(60 F254), CC, PTLC, Perkin-Elmer 65FT infrared spectrometer, GC-MS (Clarous 600), MS (Clarous 600), NMR, UV-Visible Spectrophotometer, rotary evaporators, column apparatus.

3.3 Chemicals

Petroleum ether, dimethylether, hexane, chloroform, ethylacetate, methanol, ethanol, silica gel (60 (Merck) particle size of 0.63-0.200 (70-230 mesh ASTM), distilled water, anhydrous sodium sulphate, were the chemicals that were used during the study.

3.4 Essential oil extraction from *C. ambrosioides*

100 g of fresh aerial part of *C. ambrosioides* was taken and homogenized with a kitchen juicer and then added in to 1L round bottom flask. Then 400 ml distilled water was added and the flask was fitted with essential oil extraction apparatus (Clevenger apparatus). The content was heated and essential oil was condensed with vaporized water in the part of the apparatus where condensates can be collected. As the water and essential oil are immiscible, the oil was seen floating at the upper layer of the condensate.

The two layers were separated, by taking the water (the lower layer) through the tip of the collector by opening the stop cock and the essential oil was also taken in separate rail. The water passed with the oil was dried with anhydrous Na_2SO_4 to which about 10 ml of chloroform was added.

3.5 GC-MS Analysis of the Essential Oil

The oil was dissolved in chloroform and injected to GC/MS at programmed temperature as follows. Initial temperature 50 °C-0.0 min at rate 5°C/min 250°C-0.0 min the flow rate 1ml/min injector, temp.250°C the total run time for this method was 40 min.

The sample was injected three times at different dilution to obtain good chromatogram which was well resolved. For each peak (TIC) in the chromatogram its mass spectrum was compared with similar mass spectra that were searched from NIST library of mass spectra database for identification based on retention index and spectral similarities. Thus for each peak in the sample chromatogram, five probable compounds which have spectra near to the unknown were semi-automatically selected. From the five probable compounds one with the best fit in terms of retention index and spectrum was selected.

3.6 Solvent Extraction of the Plant

The powdered aerials part of the plant material was passed through a sieve and stored in a desiccators. The air dried and powdered plant material (200 g) was first soaked with 2L methanol for 72 hours with occasional shaking. Then the material was filtered by using filter paper and the solvent was evaporated under vacuum on a rotary evaporator and a crude methanol extract was obtained (24 g). The methanol extract was further fractionated by successively washing it with n-hexane, chloroform, ethyl acetate and the extracts were collected after the solvents were evaporated under *vacuum* using the Rotary evaporator and afforded 24g, 9g, 0.69g and 0.4g brown, deep green, deep green and green solid residues, respectively, which on TLC analysis using a solvent system acetone: chloroform (7:3) showed a green yellow and red colored spot under UV light, respectively.

3.7 Preliminary phytochemical screening

Methanol extract of the whole aerial part of *C. ambrosioides* was subjected to preliminary phytochemical screening tests for various phytoconstituents following standard methods.

3.7.1 Test for alkaloids

1.5 g of mercuric chloride was dissolved in 60ml of distilled water and 5 g of potassium iodide was dissolved in 10 ml of water. The two solutions were diluted to 100 ml with distilled water. 1.2 ml of the methanol extract was taken in a test tube and 0.2 ml of dilute sulphuric acid and 0.1

ml of Mayer's reagent were added to the extract. Formation of yellowish colored precipitate was observed.

3.7.2 Test for tannins

To 5 ml of methanol extract, a few drops of 1% lead acetate were added. A yellow precipitate was formed.

3.7.3 Test for saponins

1 ml of methanol extract was treated with 1% lead acetate solution. No formation of white precipitate was observed.

3.7.4 Test for Phenol

To 5 ml of methanol extract, two drops of 1% ferric chloride was added. Formation of blue green colored solution was observed.

3.8 Column chromatography

The dry packing method was used in preparing the silica gel column. The chloroform crude extract (0.69 g) was adsorbed on silica gel and applied to the top of the column which was packed with chloroform. The column was eluted using the following solvent systems; chloroform (100%): fraction (1-2), chloroform: acetone (9:1): fractions (3-4), chloroform: acetone (8-2): fractions (5-6), chloroform: acetone (7:3): fractions (7-8), chloroform: acetone (6-4): fractions (9-10), chloroform: acetone (5-5): fraction 11-12, acetone (100%) fraction (13-15). Totally 15 fractions were collected. All fractions were analyzed by using TLC and visualized under UV light (see section 3.8). All fractions from 9-12, showed three spots on TLC using solvent system chloroform: acetone (7:3). These fractions needed extra separation and after the removal of the solvent using the rotary evaporator were combined.

Figure 9: Column chromatography of the chloroform crude extract

3.9 Thin layer chromatography (TLC) Analysis of the fractions

The content of each beaker were spotted on pre-coated silica gel on aluminum plates and developed in a small chromatographic tank to separate the different components based on their relative mobility's in solvent systems and color reactions with ultra-violet light. The amounts of the different fractions were determined. A strip of the pre coated silica gel was cut out and a spot of the sample was applied on the plate about 1.0 cm from the edge. It was dried using hot air dryer. The strip was lowered into a small chromatographic gas jar containing the solvent system. The gas jar was covered with a glass lid. The solvent was allowed to ascend until the solvent front was about edge of the length of the strip. The strip was removed and dried by a hot air dryer and viewed under UV lamp at 365 nm and 254 nm to identify the fluorescing spots. The fluorescent spot was marked and then iodine vapor used. The strip was placed in hot oven at 110 °C for 5 seconds for visibility of fluorescent bands. The color reaction was recorded and the relative Retardation Factor (RF) value was calculated based on the formula described by Stahl:

$$R_f = \frac{Distance\ travelled\ by\ the\ component}{Distance\ travelled\ by\ the\ solvent\ front}$$

Table; 1 Fractions9-15 collected from CC of the chloroform extract

Fractions	Rf. Values	TLC coloration	UV 365nm and 254nm
F9	0.62	Green yellow	Observed
F10	0.17	Green yellow	Observed
F11	0.33	Green yellow	Observed
F12	0.49	Green yellow	Observed
F13	0.35	Green yellow	Observed
F14	0.43	Green yellow	Observed
F15	0.26	Green yellow	Observed

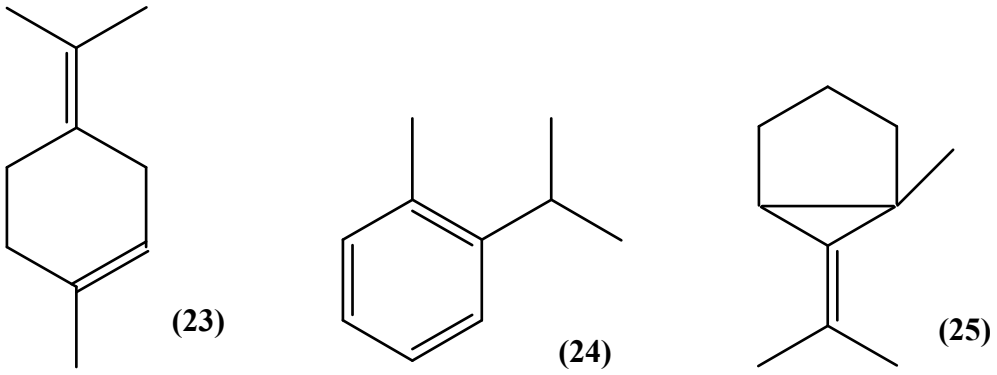
3.10. Preparative TLC Separation

The combined fractions 9-12 were developed on PTLC by using acetone: chloroform (6:4) by monitoring the separation under UV light. After repeated development of the PTLC, the major band was scraped-off from the PTLC, powdered and washed off the silica gel by methanol which gave 3 mg of CAC-1 upon removal of the methanol under reduced pressure on rotary evaporator, which moved as a single homogeneous spot under TLC.

4. Results and discussion

4.1 Result of Essential oil

Medicinal plants have been used as medicine for centuries over the world and are still in use especially in developing countries. Chemical investigation of the essential oil and solvent extraction of the whole aerial part of the medicinal plant *C. ambrosioides*, which is used for treatment of different human ailments, maintenance of health as well as treatment of mental and physical illness, has afforded one new compound and enabled the identification of three compounds from its essential oil. GC-MS of the essential oil hydro-distilled from *C. ambrosioides* afforded three major compounds; namely cyclohexene-1-methyl-4-(methlidene) (**23**), benzene-1-methyl-2(1-methyl ethyl) (**24**), 6-isopropylidene-1-methyl-bicyclo[3.1.0] hexane (**25**) (Appendix 1).



(23) **(24)** **(25)**

Figure; 10 Compounds identified form essential oil of *C. ambrosioides* by using GC-MS

4.2 Result of Solvent Extraction

200 g of dried and pulverized whole aerial parts of the plant was successively extracted using methanol, hexane, chloroform, and ethyl acetate, and the crude extracts that were obtained are given in Table 2.

Table 2: Result of extraction of the dried and pulverized aerial parts of *C. ambrosioides* using different organic solvents

S. No.	Solvent for extraction	Crude extract(g)
1	Methanol	24
2	Hexane	9
3	Chloroform	0.64
4	Ethyl acetate	0.4

4.3 Preliminary phytochemical analysis *C. ambrosioides*

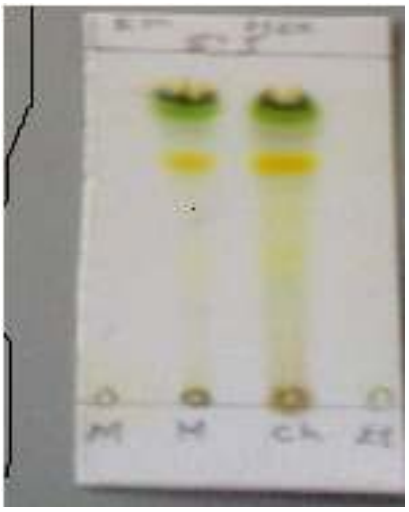
Phytochemical analysis of the plant extract indicated the presence of various natural products which included tannins and phenolic compounds but absence of saponins and alkaloid as shown in Table 5.

Table.3 Results of the phytochemical assay of the crude methanol extract of *C. ambrosioides*

Extract	Phenol	Saponins	Tannins	Alkaloid
Methanol	+	-	+	-

4.4 TLC Analysis of the Extracts

All the extracts were analyzed with TLC by using different solvent systems which included ethyl acetate: hexane (7:3) and ethyl acetate: hexane (5:5). The results that were obtained are shown in



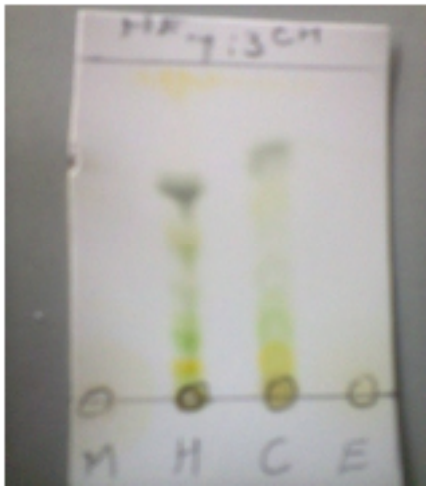


Figure 11: TLC of methanol, hexane, chloroform and ethyl acetate extracts developed by using hexane and ethyl acetate as a mobile phase hexane

4.5 Isolation of compounds by CC

0.64g of chloroform was adsorbed on 10g of silica gel and applied at the top of a column packed with 70g silica gel using chloroform. The column was eluted using the solvent systems given in Table 4.

Table 4: Fractions 1-15 obtained from CC of the chloroform extract

Solvent	**Ratio**	**Fraction**
Chloroform	100	F-1,F-2
Chloroform/acetone	9:1	F3,F-4
Chloroform/acetone	8:2	F5,F6
Chloroform/acetone	7:3	F7-8
Chloroform/acetone	6:4	F9-10
Chloroform/acetone	5:5	F11-12
Acetone	100	F13-15

After analyzing fractions 1-15 by TLC, fractions 9-12 were combined due to their similar TLC profiles. The combined fractions were further separated on PTLC and the major band was scraped-off and washed with acetone yielding 3mg of a pure compound, CAC-1.

4.6 Characterization of isolated compound

Compound CAC-1 (3 mg) was isolated using repeated chromatography from polar fractions (fractions 9-12) of the chloroform extract as described earlier. TLC analysis of CAC-1 by using a solvent system of chloroform: acetone (3:7) showed a single, homogeneous spot. NMR spectra of the compound (^{1}HNMR, ^{13}CNMR, and DEPT-135) were recorded and the structure of the compound was partially characterized as 1-(4(2-(4-(2-(2-hydroxyethoxy)ethyl)phenyl) propan-2-yl)bicyclo[1.1.1]pentan-2-yl)ethanone. The ^{1}H NMR of the compound showed two separate sets

of doublet of doublets (J= 3.6 and 2.4 Hz) centered at δ7.64 (2H) and 7.46 (2H) respectively indicating presence of a *para*-disubstituted aromatic ring in the structure. In addition, two

separate methylene triplets centered at δ4.24 and 4.15 with J=6.8 Hz and 5.6 Hz respectively, indicated the presence of two oxygenated methylenes bonded to two other open chain methylenes. Presence of a six proton singlet at δ1.18 indicated presence of two equivalent tertiary methyl groups.

The ^{13}C NMR of the compound gave carbon signals at δ198.13 due to a ketone functional group because had it been an aldehyde function, aldehydic proton signal at δ9.8 would have been observed in ^{1}H NMR of the compound. Further, the carbon signals at δ130.9 due to two olefinic quaternary carbons, 130.88 (2x), 128.84, 128.81 due to four olefinic methine carbons corroborated the presence of the *para*-disubstituted aromatic ring in the compound. Carbon signals at δ77.33, 68.17, and 65.57 which appeared as negative peaks in DEPT spectrum indicated presence of three oxygenated methylenes. Two negative signals at 38.74 and 25.42 in the DEPT spectrum indicated presence of two aliphatic methylenes. Four aliphatic methine signals appeared at δ30.58, 30.37, 29.70 and 28.93. Further, an aliphatic quaternary carbon signal appeared at δ19.18. Three methyl signals were observed at δ22.98, 23.76 and 29.08. Based on these NMR data the structure 1-(4(2-(4-(2-(2-hydroxyethoxy)ethyl)phenyl) propan-2-yl)bicyclo[1.1.1]pentan-2-yl)ethanone, has been proposed for the compound CAC-1 as shown in Fig. 12. .

Table 5: ^{1}H, ^{13}C, DEPT-135 NMR (400MHz, $CHCl_3$) spectral data for CAC-1

C-No.	^{13}C-signals (δ, ppm)	DEPT-135	^{1}H-Signals (δ, ppm)	Inference
1	130.90	-	-	Quaternary
2	128.80	positive	7.46	Ar-CH
3	128.84	positive	7.64	Ar-CH
4	130.88	-	-	Quaternary
5	128.84	positive	7.64	Ar-CH
6	128.80	positive	7.46	Ar-CH
1’	28.93	positive	1.31	-CH

2’	30.56	positive	1.74	-CH
3’	29.70	positive	1.32	-CH
4’	30.36	positive	1.45	-CH
5’	25.42	negative	1.73	- CH_2
6’	198.13	-	-	quaternary
7’	29.08	positive	1.32	-CH_3
8’	19.19	-	-	quaternary
9’	22.99	positive	1.18	-CH_3
10’	23.76	positive	1.18	- CH_3
1’’	38.75	negative	1.75	- CH_2
2’’	77.33	negative	4.24	- CH_2-O
3’’	68.17	negative	4.15	- CH_2-O
4’’	65.57	negative	3.51	-CH_2-O

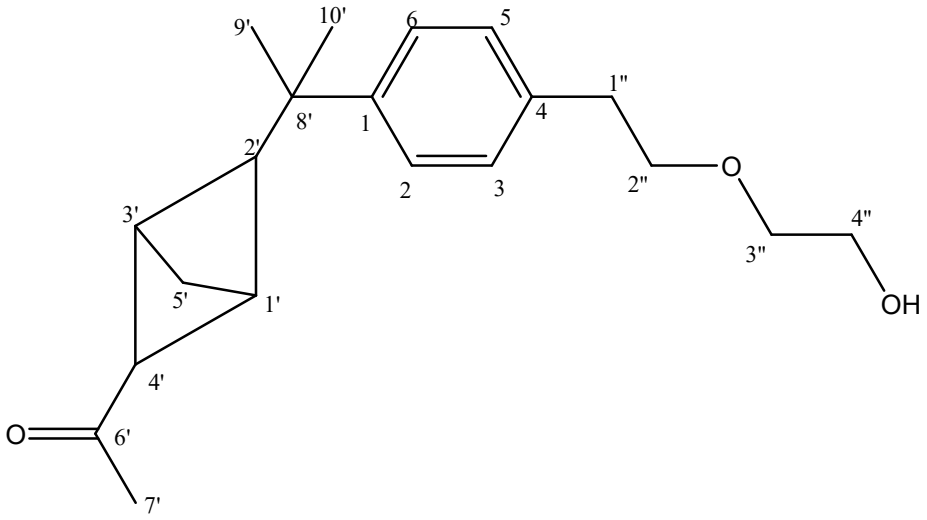


Figure 12 Proposed structure of the new compound, CAC-1

4.6.1 Spectral data

^{1}H-NMR(400MHz, $CHCl_3$): 7.52(1H, d,H-2),7.75(1H,d,H-3), 7.75(1H,d,H-5), 7.52(1H,d,H-6),4.31(2H,s,H-1``),1.75(2H,dd,H-2’),1.74(1H,t,H-3’),1.33(2H,d,H-5’) 1.32(1H,t,H-6’),1.31(1H, dd,H-7’), 1.30(3H,d,H-8’),4.22(2H, t,H-2’’), 0.99(3H,t,H-3’’)

^{13}C-NMR (400MHz, $CHCl_3$): 130.80(C-1), 128.80(C-2), 130.88(C-3), 130.90(C-4), 130.88(C-5), 128.80(C-6), 68.16(C-1'') ,65.566(C-2''), 23.760(C-3''), 198.134(C-1'), 38.74(C-2'), 30.579(C-3'), 30.37(C-4'), 19.78(C-5'), 29.70(C-6'), 28.931(C-7'), 22.58(C-8')

5. Summary, Conclusion and Recommendation

5.1 Summary and Conclusion

This study has indicated that the whole aerial part of the medicinal plant *C. ambrosioides* yields essential oil when subjected to hydrodistillation. The essential oil up-on GC-MS analysis exhibited the presence of three major compounds; namely, 1-methyl-4 (1-methyledene)-cyclohexane, 1-methyl-2 (1-methylethyl)-benzene and 6-isopropylidene-1-methyl-bicyclo [3,1,0] hexane. Further methanol extraction of the aerial parts of the plant afforded a crude methanol extract which was subjected to phytochemical screening for identification of possible classes of secondary metabolites present in the extract. Thus presence of phenolic compounds and tannins were confirmed while saponins and alkaloids were absent. Further the methanol extract was subject to successive fractionation by washing it with n- hexane, ethyl acetate and chloroform which afforded crude hexane, ethyl acetate and chloroform extracts, respectively. After TLC analysis of the crude extracts, the chloroform extract was subjected to chromatographic separations on silica gel columns eluted with chloroform and chloroform containing increasing amounts of acetone followed by preparative TLC separations. Thus, a new compound, CAC-1, was isolated from the chloroform extract of the aerial parts of the plant by repeated chromatography. The structure of CAC-1 has been elucidated by the data obtained from its ^{1}H-NMR, ^{13}C-NMR, DEPT experiments. In addition structural information about the compound has been obtained from its Infrared spectrum. By interpreting these spectroscopic data CAC-1 has been characterized as 1-(4(2-(2-hydroxyethoxyl)ethy)propanal-2-yl)bicyclo [1,1,1]pent-2-yl)-ethanone.

5.2 Recommendations

It is recommended that further study be conducted on the remaining extracts, i.e. the ethyl acetate and hexane extracts to find more of the phenolic compounds and others; as well as the methanol extract to identify the tannins that were present in the methanol extract. It will also be interesting to collect the seeds of the plant separately and investigate the essential oil of the seeds since it was the oil from the seeds that had been extensively studied elsewhere and found to contain the medicinally important compound, ascaridole, which was glaringly absent from the essential oil of this plant which this study utilized.

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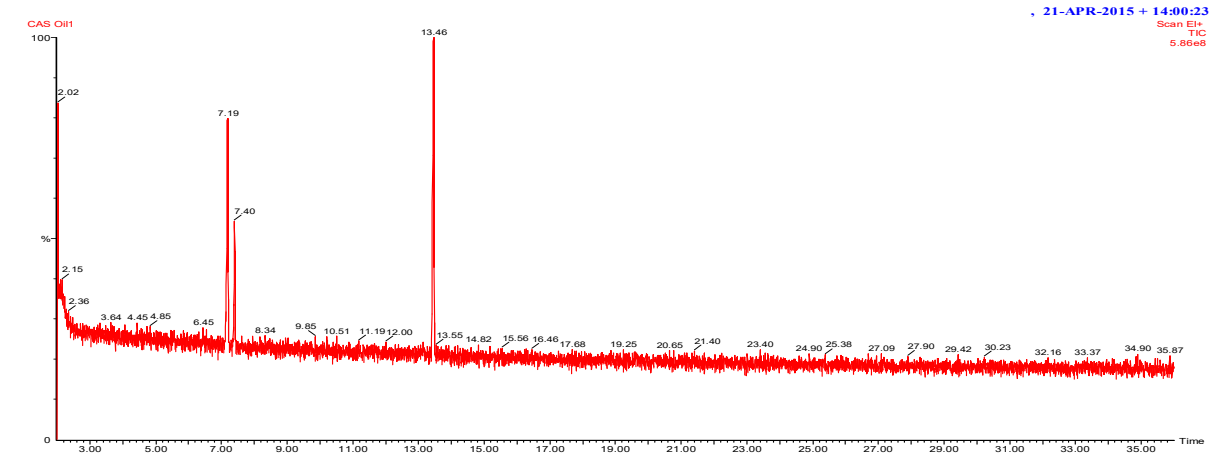
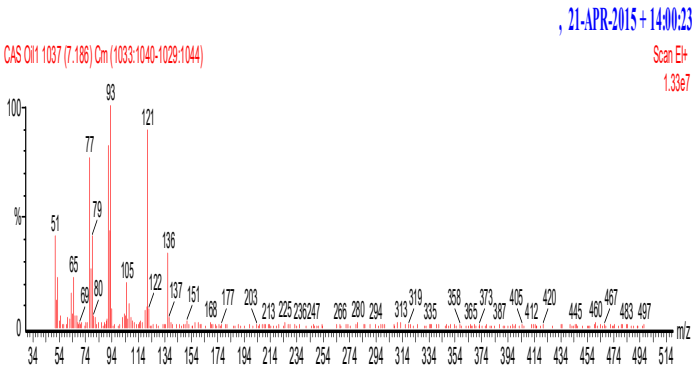
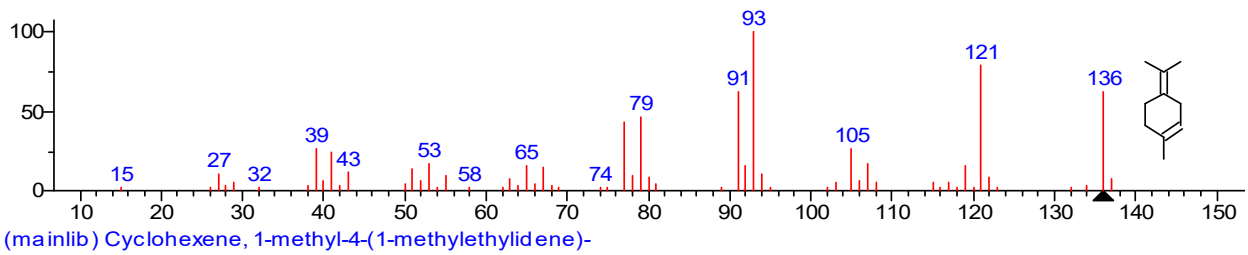
7. Appendix

Appendix 1 GC-chromatogram of aerial part of *C. ambrosioides* (CAS 1)

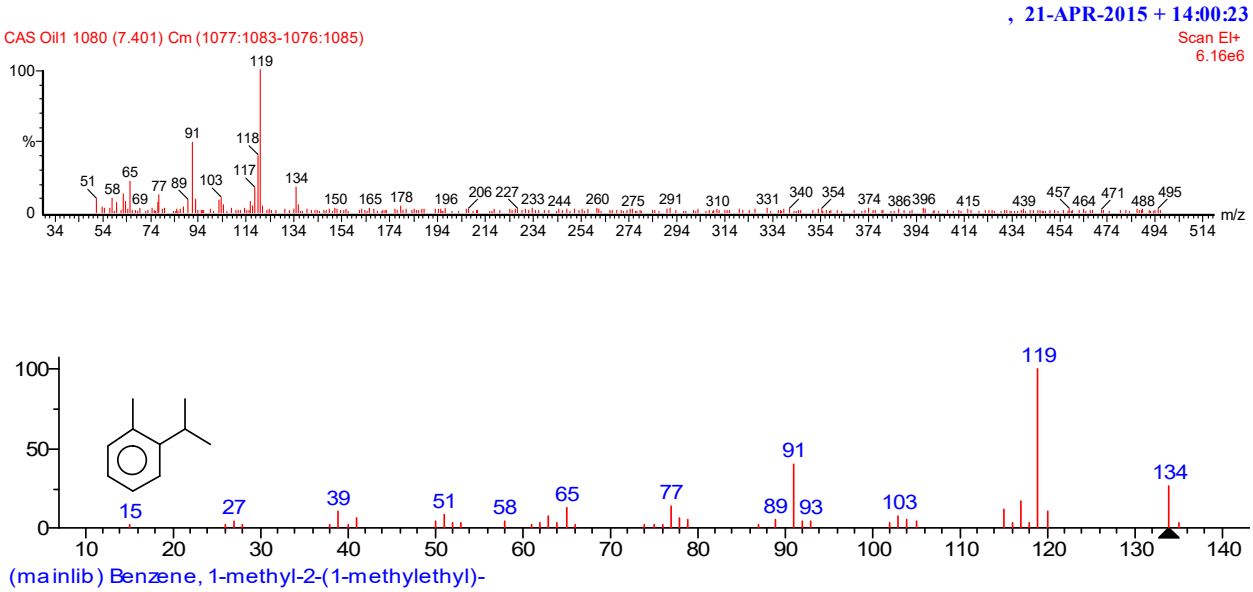


Appendix 1a

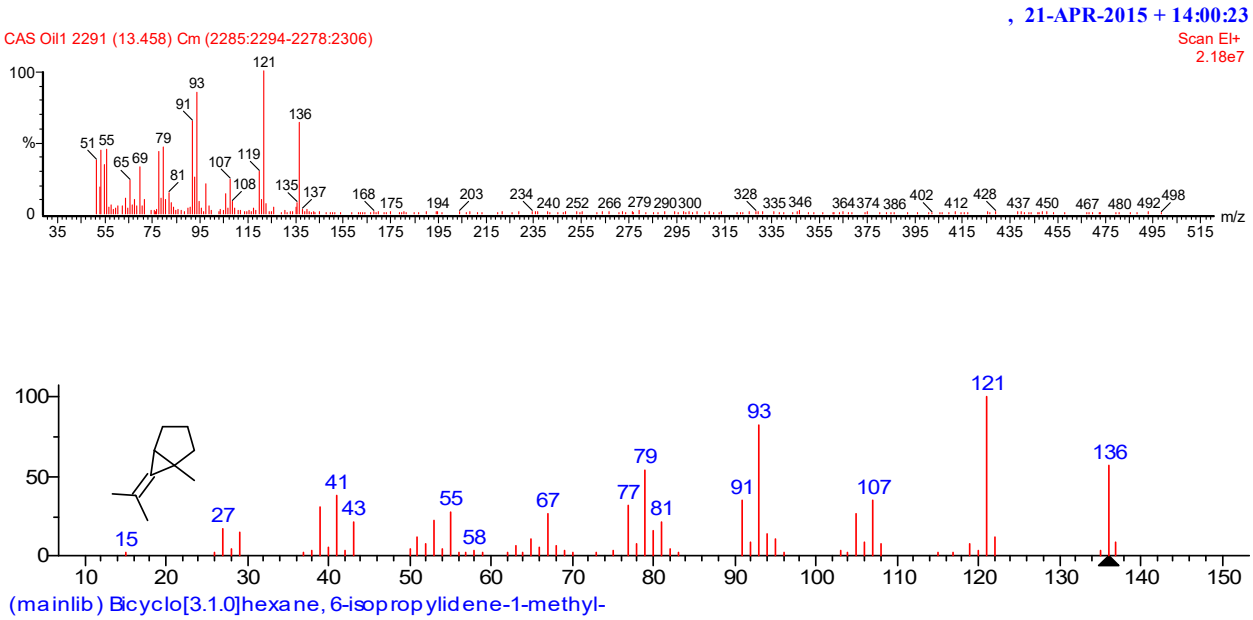




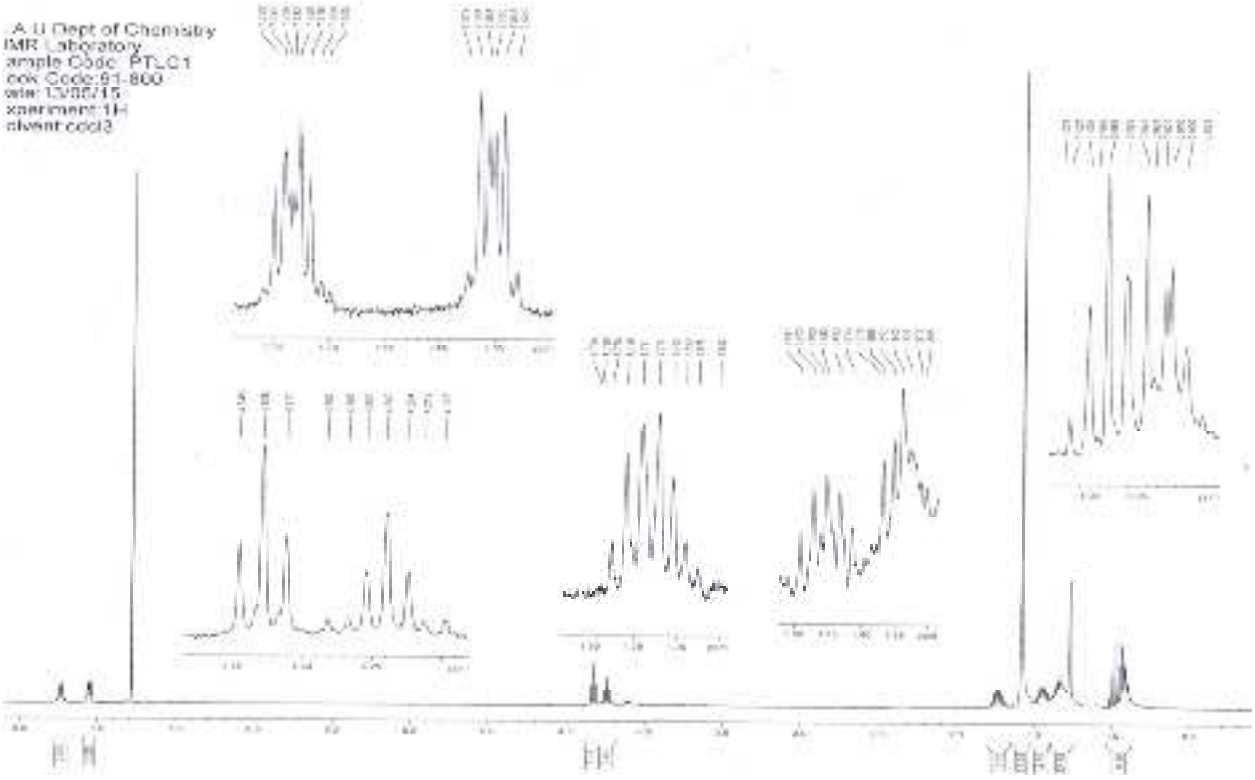
Appendix 1b



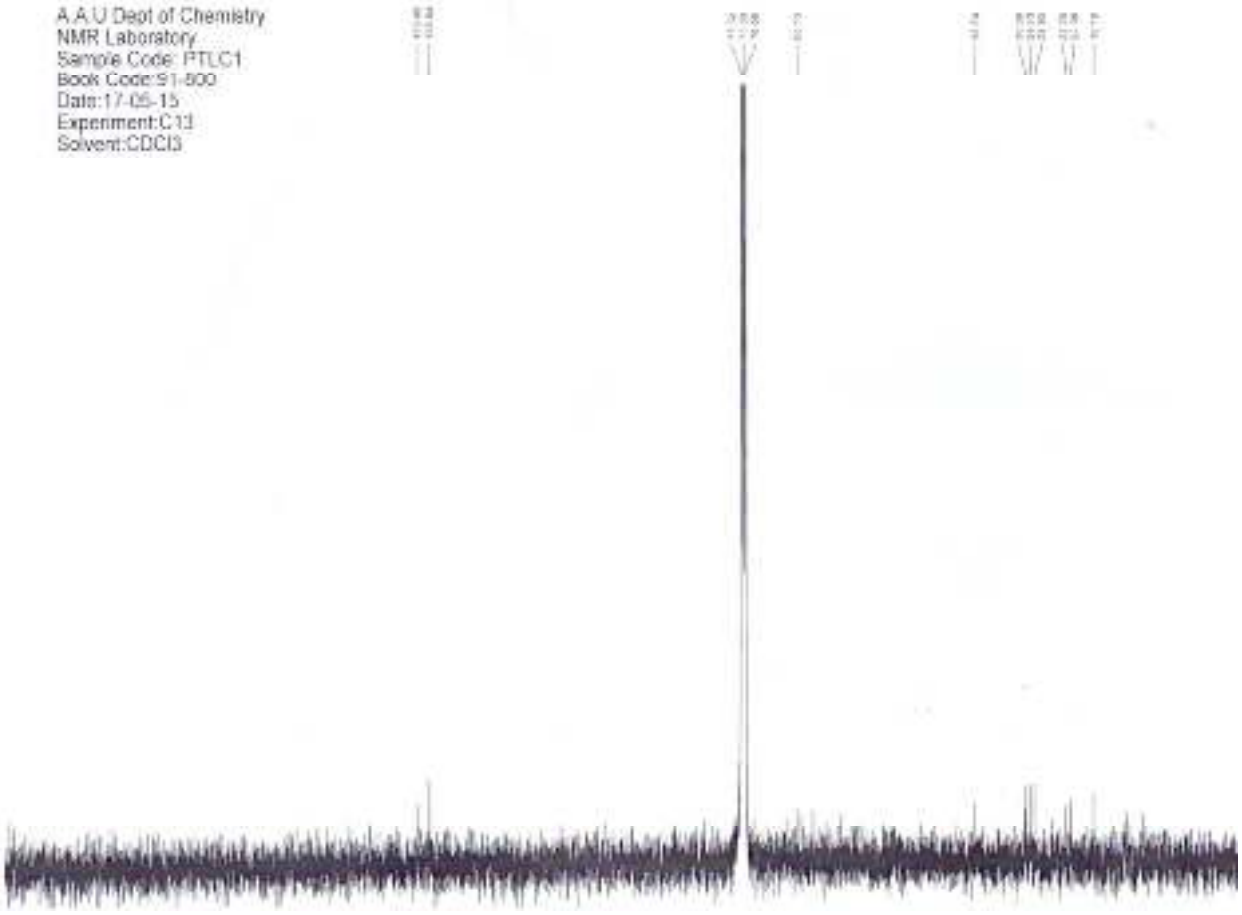
Appendix 1c



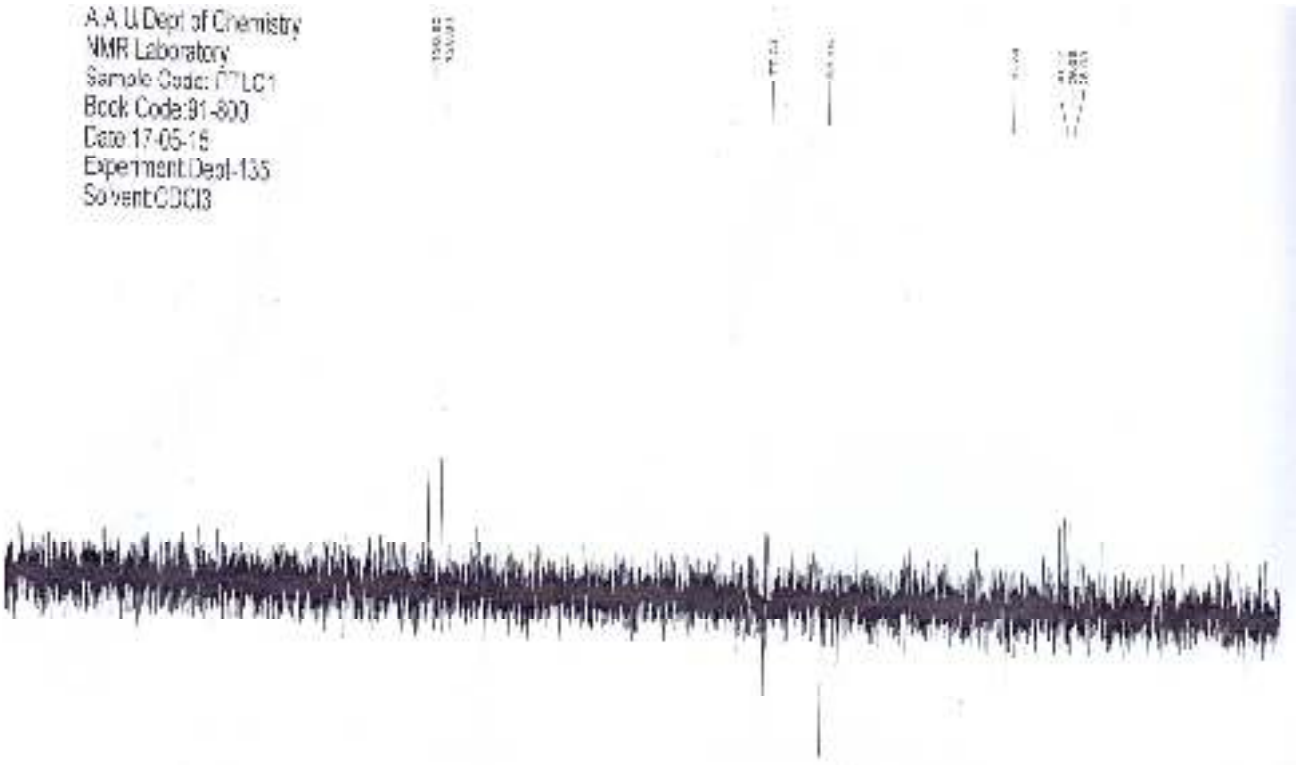
Appendix 2a ^{1}H NMR spectrum of compound CAC-1in $CDCl_3$



Appendix 2b ^{13}CNMR Spectrum compound CAC-1 in $CDCl_3$



Appendix 2c DEPT-135 Spectrum of compound CAC-1 in $CDCl_3$



Appendix 2d IR spectrum of compound CAC-1