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Characterization of some active organic compound from Cold and Hot aqueous solvent and Study their Antibiotic of *Artemisia herba-alba* Asso plant oil

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Abstract

This study includes the extraction of volatile oil from the *Artemisia herba-alba* plant (Asteraceae). Take the plant *Artemisia* from the city of heet Al-Anbar in Iraq, where the research knew the practical chemical components in the *A. herba-alba* plant. The cold and hot aqueous solvent extracted from the *A. herba-alba* plant and the study diagnoses their qualitative components. GC-mass, Thin-layer chromatography (T.L.C) used to isolate some active organic compounds from this oil. Ethyl acetate: Toluene 5: 95% used as mobile phase. The separation technique also used by Column (C.C) chromatography. Study the biological of the oil of *A. herba-alba* extracted by the cold and hot method, and studied its effect on some bacteria were. Results obtained are that plant *A. herba-alba* is acidic because it contains many compounds, phenol, resins, flavonoids, alkaloids, and turbines. GC-mass obtained got to show accompanying mixtures, camphor, filifolide A, 1-ethynyl-4-methoxy-4-methyl-2-cyclobuten-1-ol, lineatin, (furanethanol 5-ethenyltetrahydro-α,β,5-trimethyl), 2-pentanoylfuran, davana ether, caryophyllene oxide, beta-Myrcene, vulgarin, trans-caryophyllene, And bis(2-Ethylhexyl) phthalate are available in the accompanying percent 5%, 6%, 10%, 47.5%, 19%, 18.5%, 6%, 8%, 7.5%, 12.5%, 9%, and 6% separately are available in *A. herba-alba* oil that extricated by cold aqueous dissolvable. Furthermore, acquired demonstrate that the accompanying mixtures, 2-methyl-4-nitroresorcinol, cis-hydroxydavanone, vulgarin, and dihydroxanthin, are available in the simultaneous percent 4%, 8.25%, 7.25%, 5.75% individually are available in *Artemisia* oil that extracted by Hot aqueous solvent. T.L.C chromate-gram appears six zones for Cold aqueous solvent extract. Three zones of hot aqueous solvent extract identified and characterized by different spectroscopic methods UV-vis. And Ft-IR. Spectra. Some of the compounds appear six zones for Cold aqueous solvent extract, and three zones hot aqueous solvent extract ethyl acetate: Toluene used as mobile phase. Obtained and diagnosed the separation compound by spectroscopy UV-Vis and Ft-IR spectra. The active organic compounds present in the extract of the *A. herba-alba* plant have proven to have high biological efficacy against bacteria.

***Keyword*:** UV-Vis, Artemisia, plant, Ft-IR, bacteria, column.

1. Introduction

Artemisia is known and separates all through the world for its remedial properties [1]. The ethereal bits of these plants used in traditional medicine as implantations, to which anthelmintic, antibacterial, antipyretic, cytostatic, stomachic, and antitumor activities have been credited [2]. For centuries, flavours and species have been used to help protect food sources [3] shield them from microbial contamination. Research shows that Artemisia contains constituents that can thwart pollution and clean heretofore spoiling nourishments[4]. Oil of Artemisia gotten from Artemisia [5]. The enduring spice, an individual from the fundamental family, is used in fragrant mending, cooking, mix, mouthwashes, and elixirs, similarly as added to demulcents [6]. Artemisia has in like manner different clinical properties, which is a result of the flavour's essential oil [7]. The clinical properties of Artemisia oils (which are separated through steam refining of new blossoms and leaves) are because of their segment Acne, Anticancer, Antispasmodic, Antirheumatic, Antiseptic, Bactericidal, tonic, genial, carminative, bug spray energizer, yeast executioner, and others[8]. The 500 types of Artemisia are generally enduring spices overwhelming the tremendous steppe networks of Asia. Asia has the most prominent grouping of species, with 150 promotions for China, 174 in the

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previous Soviet Union, around 50 detailed for Japan, 35 types of the sort found in Iran, and about 30 in Italy. Artemisia species are often used to treat various sicknesses, for example, jungle fever, hepatitis, disease, aggravation, and contaminations by growths, microscopic organisms, and infections[9,10]. It is also currently used as an experimental treatment around the world to treat Covid-19[11]. When volatile constituents from various populations of A. herba-alba obtained in different locations in Israel were compared, there was a lot of variation. [12]. Chrysanthenyl acetate was the most abundant component in A. herba-alba samples obtained at Elat (31%), followed by chrysanthenol (6.4%) acetophenone xanthocyclin. The essential oil of A. herba-alba from the Judean desert exhibited 1,8-cineole as the primary compound (50%) followed by appreciable amounts of α- thujone and β-thujone (27%) and other oxygenated monoterpenes such as terpinen-4-ol (3.3%), camphor (3%) and borneol (3%) [13, 14]. Essential oils of Artemisia herba-alba obtained in other parts of Israel contained several rare volatile terpenes, such as Artemisia alcohol and lyratol. [15]. Also discovered were two oil types for plants grown in Israel and Sinai, the cineol-thujane-bornane variety and the pinane type, with 1,8-cineole in differing concentrations in the oil of all studied populations. In more modern times, It was discovered that due to differences in the major components of the oil, a further five chemotypes could be distinguished in plants growing in the Holy Land and Sinai, implying that there are more chemovarieties in this area than previously thought [16]. Furthermore, Artemisia ketone has been identified as the primary component of an Egyptian chemotype. In contrast, 1,8-cineole, camphor, and chrysanthenone were more prevalent in the French type. [17,18]. Thus, this study aimed to use chromatographic methods, such as column chromatography (CC) and thin-layer chromatography, to extract fractions of different phenolic compounds from A. herba-alba increasing in Iraq (T.L.C.). The disc diffusion assay and T.L.C. bioautography assay were used to assess antimicrobial activity against bacterial pathogens and verify the inhibitory impact of extracted phenolic compounds. The novelty of this study is that it describes for the first time the use of Soxhlet apparatus for the extraction of phenolic compounds from the A. herba-alba. The principal objective of this work was to Essential oil Extraction, isolation, and analysis by GC–MS., T.L.C, C.C and study antibiotic of A. herba-alba plant oil.

2. Experimental

The plant A. herba-alba was obtained from the desert of Al-Anbar in Iraq in august 2020 is the collection, it is identified by the Desert Studies Center - Herbarium) - University of Anbar

Family: - Asteraceae Type: - Artemisia Herba-Alba Gender: - Artemisia

Arabic name: - Sheeh Order : - Asterales English Name: - wormwood

Common names: - compositae Class :- Dicotyledones Division : - Spermaphyta

Subdivision : - Angiospermae [19], it was air dried and packed in plastic containers.

2.1 Extraction of A. herba-alba oil by two methods:

A- Cold Aqueous solvent

500 cm3 of distilled water was applied to 50 gm of A. herba-alba plant in one litter, a round-bottomed flask. The extraction process was carried out for 96 hours, and then the volatile oil was separated from the aqueous phase with ethers and then dried with Na2SO4 anhydrous. The filtrate evaporates in the water bath at 38 oC. The oils obtained were stored in dark battle.

B- Hot aqueous solvent

In 1 litter, a round-bottomed flask, 500 cm2 of distilled water, added 50 gm of A. herba-alba plant. The extraction process carried out for 6 hours in a soxhlet extractor, and then the volatile oil was isolated from the aqueous phase with ethers and then dried with Na2SO4 anhydrous. The filtrate evaporates in the water bath at 38 oC. The oils obtained were stored in dark battle[20]. Statements were made to identify the quality of the chemical components of the plant under study. The following tests were done on the cold and Hot aqueous A. herba-alba plant extracted to diagnose their qualitative components.

PH measurement

10 gm of A. herba-alba plant powder was mixed with 50 ml distilled water and stirred for 20 minutes, the mixture was filtered off, and the pH value of the filtrate was determined[21].

Test Carbohydrates To test the solution of the A. herba-alba plant, add 1 ml of alpha-Naphthol alcoholic, then a few drops of concentrated sulfuric acid through the side of the test tube, violet colour was obtained.

Test Resins 50 ml of A. herba-alba plant was mixed with 100 ml of acidified distilled water by hydrochloric acid H.C.L.; turbidity of solution refers

to the presence of Resins compounds in A. herba-alba plant extract[22].

Test Tannins Boiled 10gm of the A. herba-alba plant powder mixed with 50 ml distilled water for 5 min; the mixture was filtered off and left to cool at room temperature: Two methods were adopted to indicate the presence of tannins:

A. 1% lead acetate solution Pb(CH3COO)2 was added to 10 ml of the filtrate A. herba-alba, resulted in white gelatin precipitate; this corroborates the presence of tannins.
B. 1% Ferric chloride FeCl3 was added to 10 ml of the filtrate A. herba-alba plant, greenish-blue colour comes out; this corroborates the presence of tannins [23-24].
C. Test Saponins

a- The aqueous solution of the plant powder was agitated hardly in the test tube; the formed bulky foam refers to the Saponins when it stays for a long time.

b- 3 ml of mercuric chloride (II) solution was added to 5 ml of the aqueous extract, and a white precipitate was formed, which refers to the Saponins[22].

Test Alkaloids A. Preparation of reagents - Wagner test: 0.2gm of potassium Iodide was added in 0.127gm of Iodine in 10ml distilled water. - Mayer test: 0.135gm of Mercuric chloride and 0.5gm of potassium iodide were dissolved in (10) ml distilled water.B. Testing A few drops of 4% Hydrochloric acid solution acidified 10 ml of aqueous extract; 1ml was taken from the solution and placed in watch glass then the above reagent was added; [25-26].

Test Glycosides 5 ml from aqueous extract plant A. herba-alba plant was mixed with 5 ml of Fehling's solution, heating the mixture for 12 minutes in a water bath, or mixed with 5 ml of Benedict's solution; the red precipitate appearance in both tests is evidence for the presence of Glycosides. [22].

Test Flavonoids 10 ml of (70% Ethanol) was mixed with 10 ml of (50% potassium hydroxide). 5 ml of Ethanoic acid extract was mixed with 5 ml of the above solution, and the colour turned yellow, which indicates the presence of Flavonoids. [27].

Test Coumarins A. herba-alba plant of Ethanolic acid extract was placed in a test tube, covered by a filter paper moistened with diluted NaOH, which was placed in a boiling water bath for a few minutes[28]. The filter paper turned yellowish-green while putting under the radiation of UV-Vis source, which refers to the presence of Coumarins.

Test proteins Biuret test is a general test for compounds having a peptide bond. Biuret is a compound formed by heating urea to 180 C. When biuret is treated with dilute copper sulfate in alkaline conditions, a purple-coloured compound is formed. This is the basis of the biuret test widely used for the identification of proteins and amino acids. Biuret reagent, i.e., a mixture of hydrated copper sulfate, potassium hydroxide solution, and potassium sodium tartrate. Two ml from aqueous extract plant A. herba-alba added to 2 ml of Biuret test Violet colouration is evidence of the presence of proteins[29].

Test terpenes 3 ml from aqueous extract plant A. herba-alba added to 3 ml Ethanol and 3 ml chloroform and added six drop anhydride acid and six drops of sulfuric acid. The appearance of a brownish colour is evidence of the presence of terpenes in the extract of the A. herba-alba plant.

Test steroids by using Liebermann Burchard reagent; each sample was put into a test tube, then dissolved in 0.5 mL chloroform and 0.5 mL acetic acid anhydrous. The mixture is then added to 1-2 mL of sulfuric acid in the tube wall, and if a bluish-green is formed, it indicates the presence of steroid compounds in the extraction and hydrolysis-partition results.

- 2 ml from aqueous extract plant A. herba-alba added to 2 ml Ethanol and 2 ml chloroform and added four drop anhydride acid and four drops of sulfuric acid. The appearance of blue colour indicates the presence of steroids in the extract of the A. herba-alba plant[30].

2.2 Analysis Essential oil by chromatography methods

Gas Chromatography and mass analyzer

The identification of the compounds in the essential oil made according to Serap et al. [31] methods recorded in the University of Mustansiria, College of science using Schimadzo GCMS-QP2010 Ultra with a flame ionization detector. A thermon-600T fused silica (50m X 0.25 mm I.D.) film thickness 0.25 µm to 60 µm long and capillary column coated with o. 3µm layer of macrogol 20,000 used. The carrier gas has nitrogen at a flow rate of 2 ml/min.; On the Column Flow, both the injection and detector temperature were 240°C. Kept the oven temperature column at 70°C for 10 min., programmed to rise to 180°C at a rate of 2°C/min., and then kept constant at 180°C for 30 min. Pressure: 100.0 KPa. Start Time: 4.50min End Time: 27.00min. Identified the essential oil components by comparing their retention times with those of authentic samples. Gas Chromatography

used for the detection of the compounds quantitative and qualitative compounds in the A. herba-alba plant. While mass analyzer detection about molecular weight of the active compound in A. herba-alba plant.

Thin-layer chromatography:

From different solvents, we choose the mixture of ethyl acetate and toluene in percent 5% and 95%, respectively, as a mobile phase, while suitable plastic paper coated with silica gel was selected as a stationary phase[32]. For testing the essential components of the volatile oil A. herba-alba plant that extracted by Cold and Hot aqueous solvents in the laboratory, the following solvents have been used as the mobile phase of T.L.C:

EtOAc: Toluene (0.7:9.3) EtOAc: Toluene (0.5:9.5) EtOAc: Toluene (1.0:9.0)

Silica gel as a coating material of about 0.25 mm crystal diameter was used to make the preparative layer chromatography of the dimension (15 cm X 5 cm). The thickness of T.L.C 1 millimetre [33].

Column chromatography

In the present study, Cold and Hot aqueous solvent of A. herba-alba plant oil subjected to column chromatography, the elution was done using toluene followed by toluene: ethyl acetate with increasing polarity and the fractions obtained were tested with T.L.C. [34].

UV-VIS.spectra were recorded using Schimadzo UV-VIS spectrophotometer 715 over the range 260-500 nm, using D.C.M. as a solvent, to analyze the compounds that have been isolated in this study from the preparative T.L.C.

Ft-IR spectra were recorded at the University of Anbar College of science using Schimadzo spectrophotometer over the range 600-4000 cm-1 to analyze the compounds that have been isolated[35].

2.3 Study the effectiveness of antimicrobial Antibacterial

In this part of the research, study the effect of the active organic compounds in the aerial amount of A. herba-alba oil on the growth of three species of bacteria, two negative dye-gram and one positive to dye-gram: (Staphylococcus aureus, Pseudomonas aeruginous, and Escherichia coli). was obtained, bacterial isolates were diagnosed from children and women live in Iraq by the Department of Biochemistry - College of Science - University of Anbar), as used diffusion method on drilling on agar dishes to test the effectiveness of the isolated compounds as shown by[36] the following steps: -

2.14.1.1 Preparing the culture media

Attended the media culture (Muller Hinton agar) by dissolving 38 grams of agar per litre of distilled water to make it more convenient to work in the production of diameters control positions within the limits of proliferation. Sterilization culture media for the culture media is used in transplant bacteria completely free of any contaminants, sterility before use temperature of 120°C for 15 minutes. Casting dishes Cold, sterile culture media, then poured in the dishes and left the dishes in the atmosphere of a room to the center solidifies culture. Activate bacterial isolates made bacteria in temperature 37°C in an incubator and dissemination stuck using a wire loop sterile on the roof of the center of the test and then left the dishes for two 20 minutes to lead to proliferation and drought. Planting of dishes Worked digging a diameter of 6 mm on the surface of culture media by sterile absorbent, and put 10 µL of the active ingredient extracted from the aerial part of A. herba-alba plant and dilute in concentrations with DMSO solutions: (25, 50, 75, 100) v/v. Incubator culture media Put the culture media, which was fertilized in an incubator, and put it at temperature 37°C, which the appropriate class for the growth of bacteria for 24 hours. Measuring inhibition zone (diameter of inhibition) Zone pellucid surrounding the pit was used to estimate the diameter hole including where bacterial growth, called a diameter of inhibition, did not happen (Zone of Inhibition) and by using the ruler listed in millimetres.

3. Results and discussion:

The qualitative laboratory analyzes. This investigation of an aerial plant of Artemisia, compound constitution, and physiochemical constitution of unpredictable oil acquired from airborne pieces of A. herba-alba gathered from west of Iraq. This plant was characterized at the University of Al-Anbar, the Center for Desertification Studies. Through this investigation, it was discovered that the A. herba-alba plant Herba Alba contains significant gatherings of high advantage, in a table (1-1) which are the mixes of flavonoids, Saponins, alkaloids, starches, tannins, and phenols present in the cool fluid plant concentrate of Artemisia. Concerning the hot aqueous concentrate of the A. herba-alba plant, it contains flavonoids, Saponins, starches, tannins, and phenols, demonstrating that the chilly fluid concentrate is superior to the hot concentrate in that it includes more synthetic mixtures in the primary research facility articulations. Also, the table shows

the chemical analysis of the active ingredients of the cold and Hot aqueous solvent oil A. herba-alba plant.

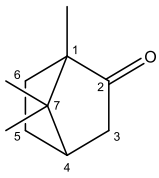
Table (1-1) Shows the chemical analysis of the active ingredients of the cold and hot aqueous solvent oil *A. herba-alba* plant.

Compounds	Reagent	Detection	Hot	Cold
Carbohydrate	Molisch's test	Violet Ring	+	+
Resins	Hcl	Turbidity	_	_
Tannins	1%Lead acetate	White gelatin precipitate	+	+
	1%Ferric chloride	Greenish blue colour	+	+
Saponins	No reagent	Large foam is formed on agitation of the aqueous solution	+	+
	Mercury II chloride	White precipitate	+	+
Alkaloids	Wagner reagent	Red precipitate	-	+
	Mayer reagent	White precipitate	-	+
	Picric acid	Yellow precipitate	-	+
Glycosides	Fehling's reagent	Red precipitate	+	+
	Benedict's reagent	Red precipitate	+	+
Flavonoids	Ethanol + K.O.H.	Yellow colour	+	+
Coumarins	NaOH then U.V. source	Greenish blue colour	_	_
Phenols	1%Ferric chloride	Green	+	+
Proteins	biuret test	Yellowish green	_	_
Terpenes	Ethanol +chloroform + sulfuric acid	Brown	_	_
Steroids	Ethanol +chloroform +anhydride acid + sulfuric acid	Yello	_	_

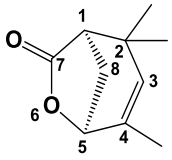
Note: (+) means that there is an active compound.

GC-mass

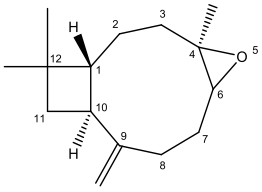
Several compounds were obtained by diagnosing the cold and hot model in the G.C.- mass spectrometric separation technique. In the cold extract of the A. herba-alba plant, 12 compounds were obtained. As for the hot extract, four compounds obtained. The results of GC/MS techniques got to show that the accompanying mixtures, camphor, filifolide A, 1-ethynyl-4-methoxy-4-methyl-2-cyclobuten-1-ol, lineatin, (furanethanol 5-ethenyltetrahydro-α,β,5-trimethyl), 2-pentanoylfuran, davana ether, caryophyllene oxide, beta-Myrcene, vulgarin, trans-caryophyllene, And bis((2-Ethylhexyl) phthalate are available in the accompanying percent 5%, 6%, 10%, 47.5%, 19%, 18.5%, 6%, 8%, 7.5%, 12.5%, 9% and 6% separately are available in A. herba-alba oil that extricated by cold aqueous dissolvable. Furthermore, acquired demonstrate that the accompanying mixtures, 2-methyl-4-nitroresorcinol, cis-hydroxydavanone, vulgarin, and dihydroxanthin, are available in the accompanying percent 4%, 8.25%, 7.25%, 5.75% individually are available in Artemisia oil that extracted by Hot aqueous solvent.



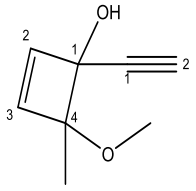
(Camphor)



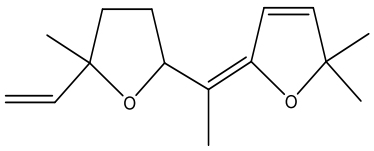
(Filifolide A)



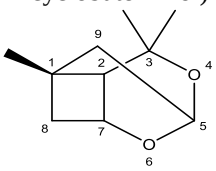
(Caryophyllene oxide)



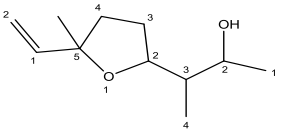
(1-Ethynyl-4-methoxy-4-methyl-2-cyclobuten-1-ol)



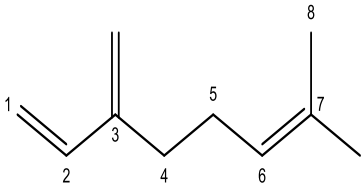
(Davana ether)



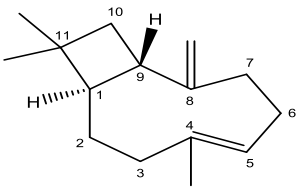
(Lineatin)



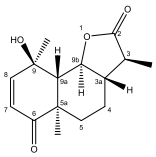
(2-Furanethanol, 5- ethenyl tetrahydro-α,β,5-trimethyl)



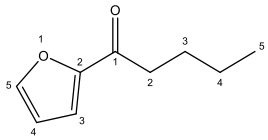
(beta-Myrcene)



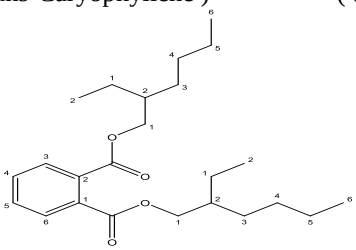
(trans-Caryophyllene)



(Vulgarin)

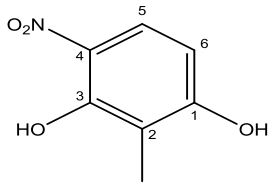


(2-Pentanoylfuran)

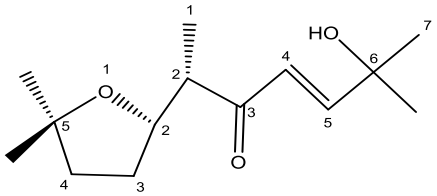


(Bis(2-ethylhexyl) phthalate)

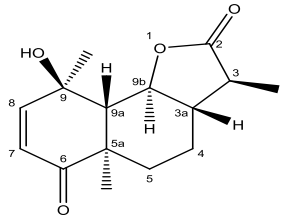
Fig. (1-1)Compounds of cold aqueous extract of oil *A. herba-alba*



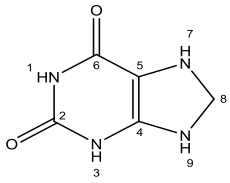
(2-Methyl-4-nitroresorcinol)



(cis-Hydroxydavanone)



(Vulgarin)



(Dihydroxanthin)

Fig. (1-2)Compounds of Hot aqueous extract of oil *A. herba-alba*

Thin-layer chromatography

For testing the essential components of the volatile oil A. herba-alba plant extracted by Cold and Hot aqueous solvents in the laboratory [37-38], the results of analysis of the volatile oil extracted by cold aqueous solvent extraction method from A. herba-alba plant. By using T.L.C revealed that oil contained, (C1) at value Rf =0.07, (C2) at value Rf =0.23, (C3) at value Rf =0.38, (C4) compound at value Rf =0.44, (C5) compound at value Rf =0.46 and (C6) at value Rf =0.51. While we get the results of the volatile oil extracted by Hot aqueous extract solvent extraction method from A. herba-alba by using T.L.C to contain (H1) at value Rf =0.07, (H2) at value Rf =0.23, and (H3) at value Rf =0.23 compound at value Rf =0.38. That is shown in Figures (3-2) and Table (3-5). The identification depends on the corresponding Rf value with the standard compound under the same conditions. The best element of separation for active compounds of volatile oil is a mixture of 0.5 volumes of ethyl acetate and 9.5 volumes of toluene; the chromatogram shows six zones in cold aqueous solvent extraction, while it shows three zones in hot aqueous solvent extraction. This system is suitable for analysis. The main reason for the difference in thinning dipole variation results is the aqueous solvent's highest dipole moment solvent. Fig(1) appear the T.L.C. test of Artemisia oil; from this chromatogram, one could observe six-zone zones characterized when compared with standard material; the results of these comparisons were explained in table(1-2)

To identify each component of this chromatogram by spectroscopic methods. Each zone was crushed, carefully isolated, and dissolved in diethyl ether, which on filtration and removal of solvent gave the desired compounds.

Tabel (1-2) Retention factor (Rf) of identification compound for volatile oil

Cold Aqueous extract		
No.	**Compound**	**Flow rate R_f**
1	**C1**	0.07
2	**C2**	0.23
3	**C3**	0.38
4	**C4**	0.44
5	**C5**	0.46
6	**C6**	0.51
Hot Aqueous extract		
NO.	**Compound**	**Flow rate R_f**
1	**H1**	0.07
2	**H2**	0.23
3	**H3**	0.38

Note: (C1) means that there first sample cold, (H1) means that their first sample hot

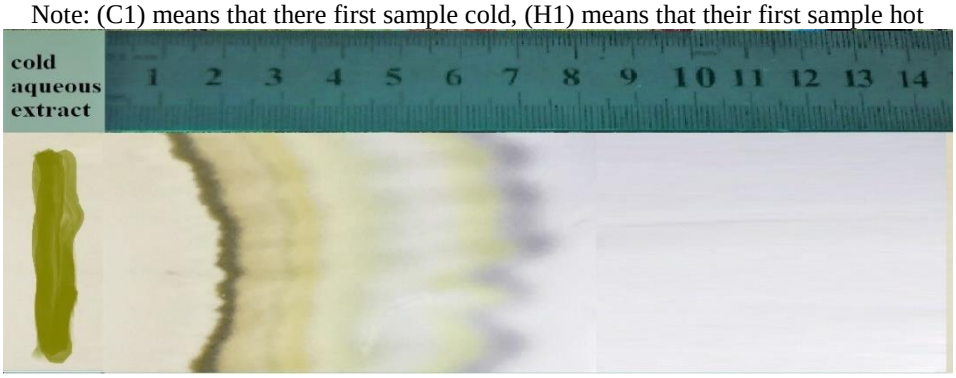


Fig (2-1) T.L.C chromatogram of Cold Aqueous extract

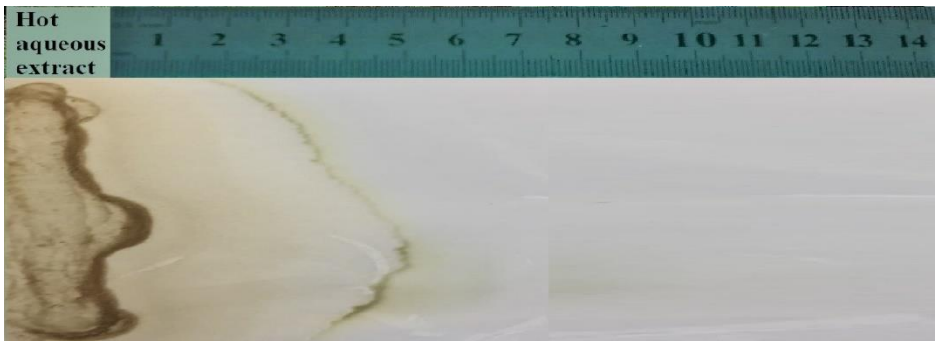


Fig (2-2) T.L.C chromatogram Hot Aqueous extract

UV-Vis spectrum of isolated compounds in Hot Aqueous extract and Cold Aqueous extract was shown in figures(2-5) and table(1-3), from there, table one could be observed that compounds The compounds separated from the hot extracted oil of A. herba-alba plant possess several influential groups. According to the results shown in UV-Vis spectroscopy, which showed absorption at certain wavelengths. The resulted are figure (C1) compound shown λmax at 266 nm and Absorbance Abs. 0.378 nm and Molar Extinction ε. 37.8 is due to the π-π* transitions, λmax 274 nm Abs. 0.415 ε. 41.5 and λmax 302 nm Abs. 0.148 ε. 37.8. Figure(C2) compound showed λmax at 266 nm Abs. 0.378 is due to n-π* and ε. 37.8, λmax 274 nm Abs. 0.415 ε. 41.5 is due to π-π* and λmax 298 nm Abs. 0.148 ε. 37.8, λmax 326 nm Abs. 0.342 ε. 34.2, λmax 340 nm Abs. 0.354 ε. 35.4. Figure(C3) compound showed λmax at 266 nm Abs. 0.044 ε. 4.4, λmax 274 nm Abs. 0.055 ε. 5.5 is due to n-π* and λmax 306 nm Abs. 0.054 ε. 5.4 is due to π-π* and λmax 306 nm Abs. 0.025 ε.2.5, λmax 306 nm Abs. 0.014 ε.1.4. Figure(C4) compound shows λmax at 274 nm Abs. 0.022 ε. 2.2, λmax 410 nm Abs. 0.014 ε. 1.4 is due to n-π* transition.Figure(C5) compound shown λmax at 266nm Abs. 0.079 ε. 7.9, λmax 276 nm Abs. 0.1 ε. 10 is due to π -π* transitions. Figure(C6) compound shown λmax at 266nm Abs. 0.071 ε. 7.1, λmax 288 nm Abs. 0.185 ε. 18.5 is due to π -π* transitions, λmax 296 nm Abs 0.186 ε. 18.6, and λmax 300 nm Abs 0.206 ε. 20.6. More of these transitions are caused by unsaturation and other active groups,

while the remaining peaks caused to affect the solvent Diethyl Ether.

As for the compounds separated from the Hot extracted oil of the A. herba-alba plant, several effective groups were also shown in U.V. spectroscopy, which showed absorption at certain wavelengths. The resulted are figure (H1) shown λmax at 290 nm and Absorbance Abs. 0.826 nm and Molar Extinction ε. 82.6 is due to the π-π* transitions, λmax 300 nm Abs. 0.905 ε. 90.5 and λmax 340 nm Abs. 0.281 ε.28.1. Figure (H2) compound showed λmax at 266 nm Abs. 0.092 is due to n-π* and ε. 9.2, λmax 272 nm Abs. 0.082 ε. 8.2 is due to π-π* and λmax 318 nm Abs. 0.048 ε. 4.8. Figure(H3) compound showed (λmax at 266 nm Abs. 0.107 ε. 10.7, λmax 272 nm Abs. 0.079 ε. 9.7 is due to n-π* and due to π-π*.

3.6.2.3 FT. I.R. spectroscopy

The most important diagnostic features of the F.T. I.R. spectra of some active organic compounds as shown in Figures (3-12),(3-13), and Table (3-9) show the spectra of all different separated compounds by T.L.C of Cold and Hot aqueous extract of the A. herba-alba plant. The compounds isolated from the cold extracted oil of the A. herba-alba plant, several effective groups also shown in Ft. I.R. spectroscopy. Resulted in the F.T. I.R. spectrum for figure (C1) compound shown revealed a band in the region 3210 cm-1 due to stretching vibration O-H group. The presence of the (O.H.) absorption band was a clear proof and a good indication of the success of the preparation reaction. 2974 cm-1 is due to C-H alkene stretching, 2858cm-1 is due to C-H stretching branched alkane, the peak at 1635 cm-1 refers to the C=C bending, 1446 cm-1 is due to the C.H. bending, 1377 cm-1 is due to O.H. bending vibration, a strong band at 1292 cm-1 is due to C-O stretching. Fig. (C2) the compound appeared in 2974 cm-1 due to alkene C-H stretching band, 2858 cm-1 is due to C-H stretching of alkane, 1782cm-1 due to C=O carbonyl group stretching, 1643 cm-1 is due to C=C symmetrical bending. 1481 cm-1 is due to CH3 symmetrical bending. 1381 cm-1 for in-plane bending C-H band, and 1293 cm-1 at a frequency range due to C-O bending.Fig. (C3) the compound showed exhibits appeared at 2936cm-1 assigned to the stretching vibration of C.H. alkene. The region 2861 cm-1 is due to stretching of C-H of alkane, 1448 cm-1 is due to C.H. bending, 1448 cm-1 is due to the C=C aromatic ring stretching. In the region 1380 cm-1, the O-H in-plane bending vibration, 1291 cm-1, is due to the C-O bending in ether. Fig. (C4) compound shows appeared in 2974 cm-1 because the alkene C-H stretching band, 2890 cm-1, is scheduled to C-H stretching of alkene, 1460 cm-1 CH3 symmetrical bending. 1310 cm-1 for in-plane bending C-O band. Fig. (C5) compound shown appeared in 2976 cm-1 is due to C-H alkene stretching band, 2862 cm-1 is due to C-H stretching of alkane, 2339 cm-1 is due to C≡C stretching of alkane, 1447 cm-1 is due to C.H. bending, 1291 cm-1 is due to C-O symmetrical bending. Fig. (C6) compound shown appeared in 2979 cm-1 is due to C-H alkene stretching band, 2867 cm-1 is due to the C-H stretching of alkane, 1687 cm-1 C=O carbonyl group stretching, 1644 cm-1 is due to C=O carbonyl group. 1448 cm-1 for in-plane bending C-H band, and 1292 cm-1 at a frequency range due to C-O bending.

As for the compounds separated from the hot extracted oil of the A. herba-alba plant, several effective groups were also shown in I.R. spectroscopy. The resulted in F.T. I.R. spectrum for the figure (H1) shown appeared in 3740 cm-1 is due to N.H. stretching, 3200 cm-1 is due to aromatic C-H stretching band, 2975 cm-1 is due to C-H stretching of alkene, 2858 cm-1 is due to C-H stretching of alkane, 1689 cm-1 is due to C=O carbonyl stretching, 1447 cm-1 is due to CH3 bending. 1377 cm-1 due to C-O. Fig. (H2) compound showed broadband, and that appeared at 3340 cm-1 assigned to the stretching vibration of the (O.H.) group. 2974 cm-1 is due to stretching of alkene, 2859 cm-1 is due to the C.H. alkane stretching. In the region, 1654 cm-1 is due to the C=O group bending vibration, 1451 cm-1 is due to the CH3 group region, a strong band 1186 cm-1 is due to C-O stretching in ether. Fig. (H3) compound showed exhibits the region 2975 cm-1 is due to stretching of C-H alkene, 2931-2858 cm-1 is due to stretching of alkane, 1442cm-1 is due to the C.H. bending. In the region, 1114 cm-1 is due to the C=C bending vibration.

3.6.3 Column chromatography

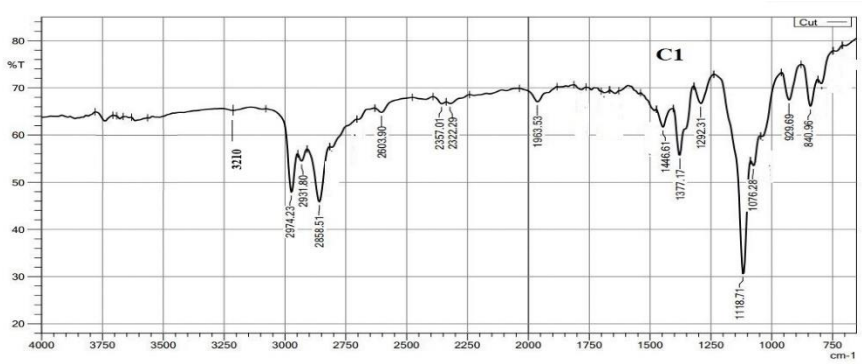
In the present study, Cold and Hot aqueous solvent of A. herba-alba plant oil subjected to column chromatography, the elution was done using toluene followed by toluene: ethyl acetate with increasing polarity and the fractions obtained tested with T.L.C. Fractions showed the same pattern in T.L.C pooled and concentrated. The polarity gradient in the separation process through column chromatography and the compounds is separated from the less polar to the more polar ones.

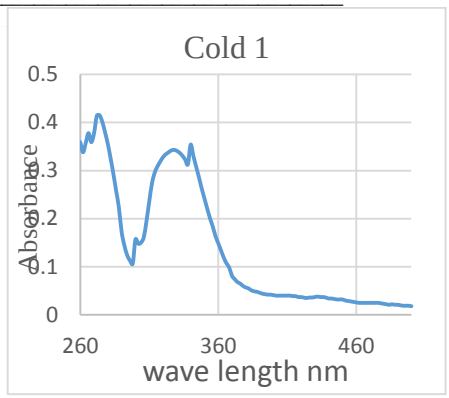
Table (1-3) UV-Visible absorption bands λ_{max} (nm) and molar extinction coefficient (ε L. mol^{-1}. cm^{-1}) for active compounds obtained from volatile oil.

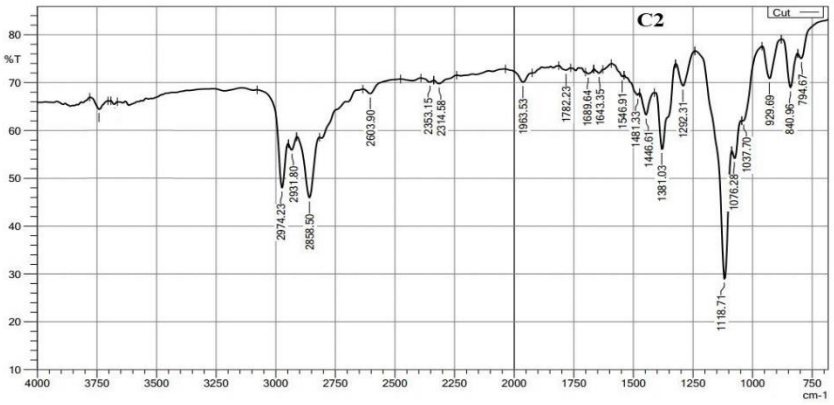
Cold Aqueous extract										
No.	**Compound**	**λ_{max} (nm)**			**Abs.**			**ε L. mol^{-1}. cm^{-1}**		
1	**C1**	266 326	274 340	302	0.378 0.342	0.415 0.354	0.148	37.8 34.2	41.5 35.4	37.8
2	**C2**	266 324	274 410	298	0.048 0.038	0.071 0.026	0.041	4.8 3.8	7.1 2.6	4.1
3	C3	266 320	204 434	306	0.044 0.025	0.055 0.014	0.054	4.4 2.5	5.5 1.4	5.4
4	C4	274	410	-	0.022	0.014	-	2.2	1.4	-
5	**C5**	266	276	-	0.079	0.1	-	7.9	100	-
6	C6	266 300	288	296	0.071 0.206	0.185	0.186	7.1 20.6	18.5	18.6
Hot Aqueous extract										
No.	**Compound**	**λ_{max} (nm)**			**Abs.**			**ε L. mol^{-1}. cm^{-1}**		
1	**H1**	290	300	340	0.826	0.905	0.281	82.6	90.5	28.1
2	**H2**	266	272	318	0.092	0.082	0.048	9.2	8.2	4.8
3	H3	266	272	-	0.107	0.097	-	10.7	907	-

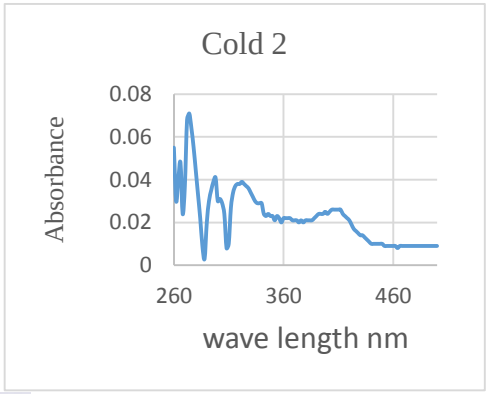
Table (3-9) determine the fraction of thin layer chromatography by Ft-IR Spectra is shown

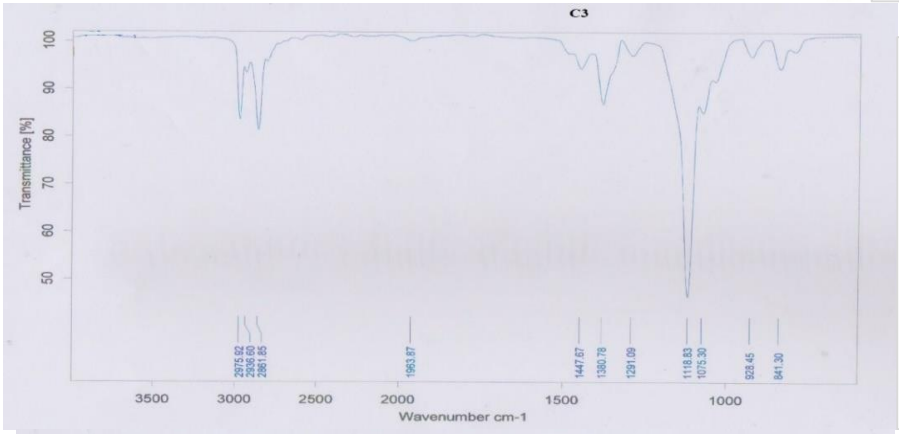
Cold Aqueous extract									
No.	**Compound**	**Assignment Cm^{-1}**							
		OH Str.	**C=C Str.**	**C.H. Str. Alk.**	**O.H. Bend**	**C-O Str.**	**C.H. Bend**	**C-O**	**C=C**
1	**C1**	3210	**2974**	2858	**1377**	-	1446	1292	1635
2	**C2**	-	2974	2858	-	**1782**	1481	1293	1643
3	C3	-	2936	**2861**	-	-	1448	1291	-
4	C4	-	2974	2890	-	-	**1460**	1310	-
5	**C5**	-	**2976**	2862	**2339 C≡C**	-	1447	1291	-
6	C6	-	2979	2867	-	1687	1448	1292	1644
Hot Aqueous extract									
NO.	**Compound**	**Assignment Cm^{-1}**							
		NH	**OH Str.**	**C.H. Str. Aro.**	**C=C Str.**	**C.H. Str. Alk.**	**O.H. Bend**	**C-O Str.**	**C.H. Bend**	**C-O**
1	H1	3740	-	3200	2975	2858	-	1689	1447	1377
2	H2	-	**3340**	2974	2859		**1377**	1654	1451	1186
3	H3	-	-	-	2975	2858-2931	-	-	1442	1114

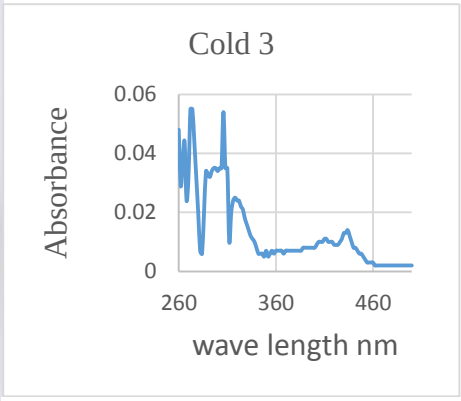

C1
Cut
%T
3210
2974.23
2931.80
2858.51
2603.90
2357.01
2322.29
1963.53
1446.61
1377.17
1292.31
1076.28
1118.71
929.69
840.96
cm-1

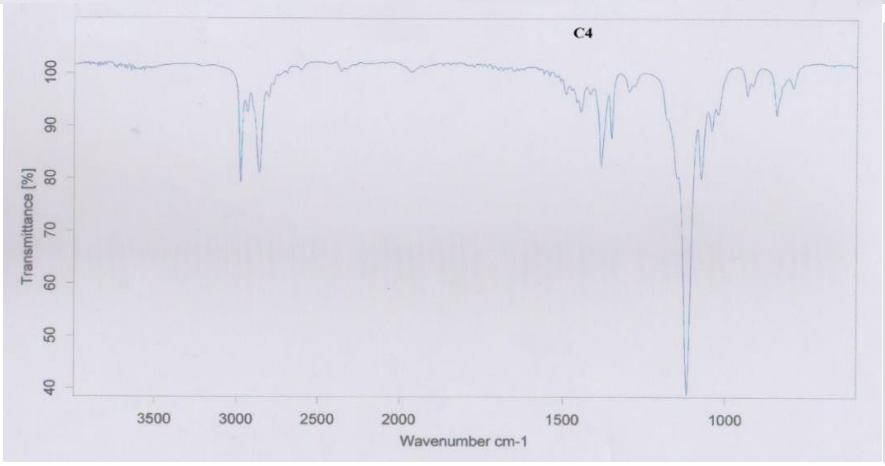

Cold 1
Absorbance
wave length nm

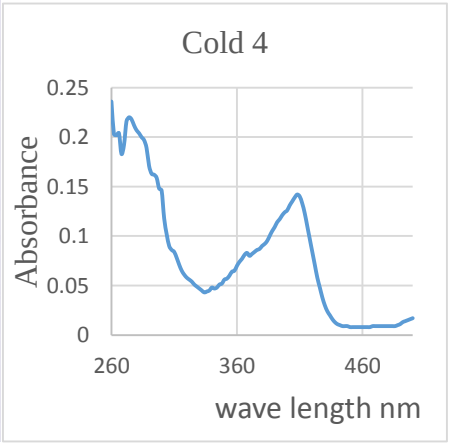

C2
Cut
%T
2974.23
2931.80
2858.50
2603.90
2353.15
2314.58
1963.53
1782.23
1689.64
1643.35
1546.91
1481.33
1446.61
1381.03
1292.31
1118.71
1076.28
1037.70
929.69
840.96
794.67
cm-1


Cold 2
Absorbance
wave length nm


C3
Transmittance [%]
2975.92
2936.60
2881.85
1963.87
1447.67
1380.78
1291.09
1118.83
1075.30
928.45
841.30
Wavenumber cm-1


Cold 3
Absorbance
wave length nm


C4
Transmittance [%]
Wavenumber cm-1


Cold 4
Absorbance
wave length nm

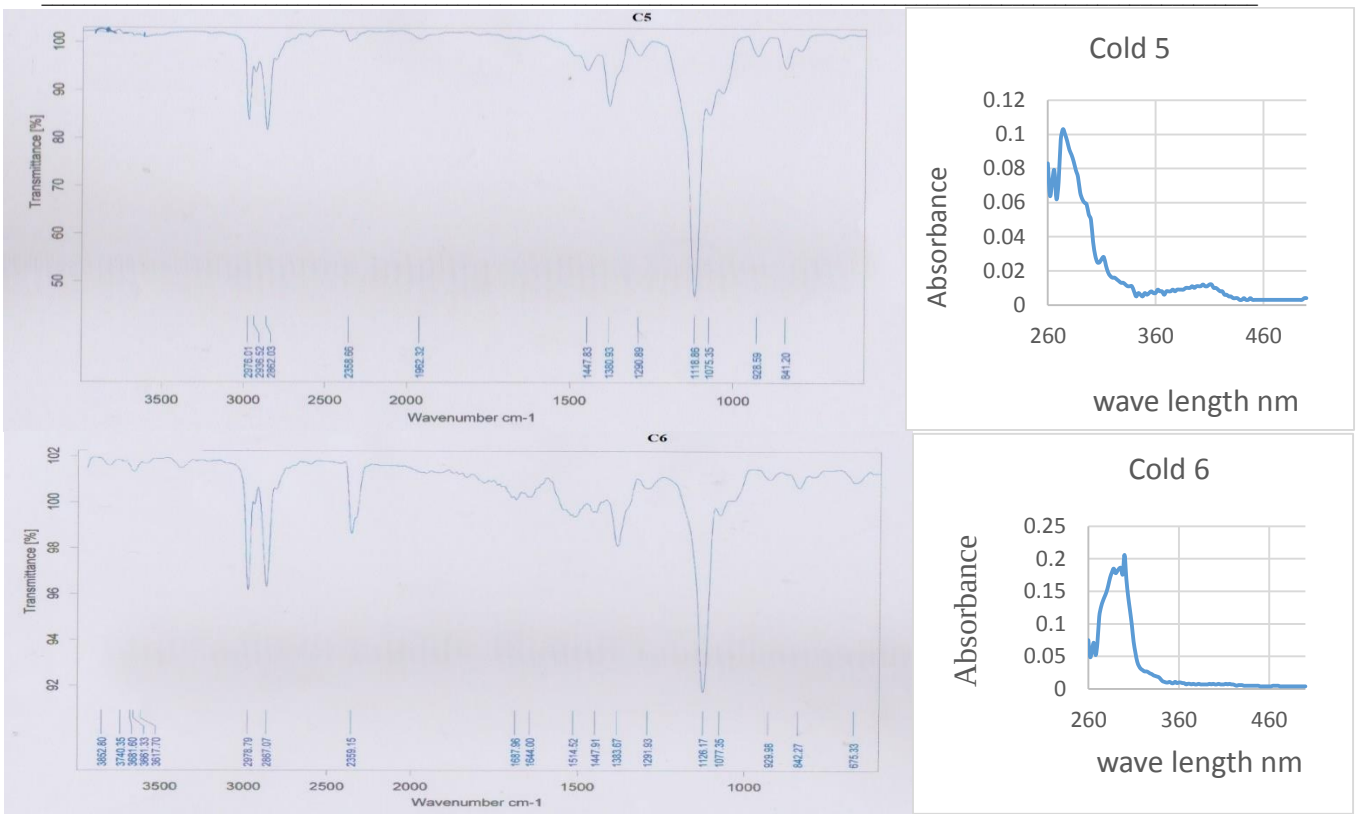


Fig. (3-1)The UV-vis and Ft-IR **spectra of active organic compounds in volatile oil that Cold aqueous extract.**

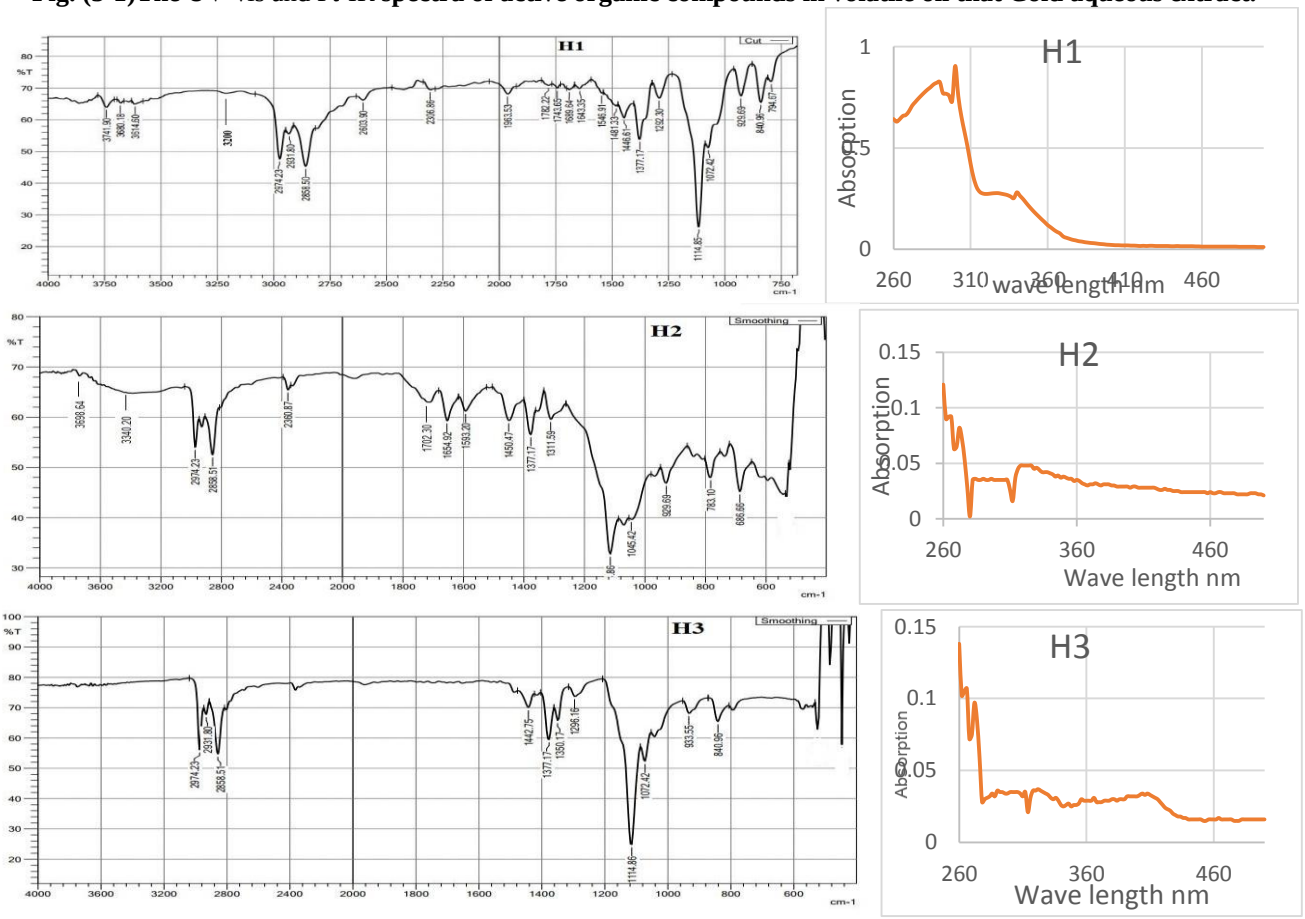


Figure (3-2) The UV-vis and Ft-IR spectra of active organic compounds in volatile oil that hot aqueous extract.

In the cold plant extract, the compound (C1) was separated by adding 4 ml of the toluene during a Retention time Rt 4 min, as for the Retention volume Rt.v (2)ml and it's colour colourless. As for the compound (C2), by adding (4) ml of toluene during Retention time Rt 8 min, as for the Retention volume Rt.v (1)ml and its colour Dark green. As for the compound (C3), by adding (3.6 + 0.4) ml of toluene + ethyl acetate during and Retention time, Rt 10 min, as for the Retention volume Rt.v (1)ml, it's colour Green. As for the compound (C4), by adding (3+1) ml of the toluene + ethyl acetate during Retention time Rt 3 min, as for the Retention volume Rt.v (1)ml and it's colour Green. As for the compound (C5), by adding (3+1) ml of the toluene + ethyl acetate during Retention time Rt 9 min, as for the Retention volume Rt.v (1)ml and its colour light green. As for the compound (C6), by adding (2+2) ml of the toluene + ethyl acetate during Retention time Rt 11 min, as for the Retention volume Rt.v (1)ml and it's colour Yellow.

In the hot plant extract, the compound (H1) was separated by adding (3.7+0.3) ml of the toluene + ethyl acetate during Retention time Rt 10 min, as for the Retention volume Rt.v (4)ml and it's colour Dark green. As for the compound (H2), by adding (3.5+0.5) ml of the toluene + ethyl acetate during Retention time Rt 10 min, as for the Retention volume Rt.v (4)ml, its colour Dark green. As for the compound (H3), by adding (2+2) ml of the toluene + ethyl acetate during Retention time Rt 5 min, as for the Retention volume Rt.v (2)ml and it's colour Yellow.

Table (3-10) shown Fractions of column chromatography that isolated from cold and hot aquouse extract volatile oil of *A. herba-alba plant*

Cold Aqueous extract					
NO.	**Compound**	**R_t volume ml**	**R_t time min**	**Solvent phase Toluene + ethyl acetate**	**Colour**
1	**C1**	2	4	4 ml + 0 ml	**colorless**
2	**C2**	1	8	4ml + 0 ml	Dark green
3	**C3**	1	10	3.6ml + 0.4ml	Green
4	C4	1	3	3ml + 1ml	Green
5	C5	1	9	3ml + 1ml	light green
6	C6	1	11	2ml + 2 ml	Yellow
Hot Aqueous extract					
NO.	**Compound**	**R_t volume Ml**	**R_t time Min**	**Solvent phase Toluene + ethyl acetate**	**Colour**
1	H1	4	10	3.7 ml + 0.3 ml	Dark green
2	H2	4	10	3.5 ml + 0.5 ml	Dark green
4	H3	2	5	2ml + 2 ml	Yellow

The composition of the fractions collected during column chromatographic separation of oil A. herba-alba plant and the concentrated fractions are shown in Table 2 were tested for determination of total by UV spectroscopy is shown in Table 3. The UV spectroscopy analysis of the fraction collected shows the percentage of total oil Artemisia plant present in the fraction. These compounds were further studied for biological activity properties. UV-vis spectra of five compounds obtained from preparative (T.L.C) recorded using t in the range of (250-700) nm. Figure (3-3) and table (3-7) shows the spectra and the absorption of all different compounds of Cold and Hot aqueous extract of A. herba-alba plant. The compounds separated from the Cold extracted oil of the A. herba-alba plant also showed several effective UV spectroscopy groups, which showed absorption at specific wavelengths.

UV-Vis spectrum of isolated compounds in Aqueous hot extract and Cold Aqueous extract shown in figures(5-1) and table (1-6), from there Table one, could be observed that compounds. The compounds separated from the hot extracted oil of the A. herba-alba plant possess several effective groups according to the results shown in UV-Vis spectroscopy, which showed absorption at certain wavelengths. The resulted are figure (C1) compound shown λmax at 278 nm and Absorbance Abs. 3.57 nm and Molar Extinction ε. 71.4 is due to the π-π* transitions, λmax 390 nm Abs. 1.02 ε. 20.4, λmax 418 nm Abs. 0.224 ε. 4.48 and λmax 645 nm Abs. 0.462 ε. 9.24. Figure(C2) compound showed λmax at 407 nm Abs. 2.26 ε. 45.2

is due to n-π*, λmax 511 nm Abs. 0.237 ε.4.74 is due to π-π*, λmax 532 nm Abs. 0.28 ε. 5.6, λmax 609 nm Abs. 0.229 ε. 4.58, λmax 664 nm Abs. 1.057 ε. 21.1. Figure(C3) compound showed λmax at 275 nm Abs. 4 ε. 80, λmax 406 nm Abs. 1.115 ε. 22.3 is due to n-π* and λmax 661 nm Abs. 0.476 ε. 9.52 is due to π-π*. Figure(C4) compound shows λmax at 283 nm Abs. 3.92 ε. 78.4, λmax 409 nm Abs.0.552 ε. 10.44 is due to n-π* transition and λmax 667 nm Abs. 0.168 ε. 3.36 is due to π-π*. Figure(C5) compound shown λmax at 273nm Abs. 4 ε. 80 and λmax 695 nm Abs. 0.063 ε. 1.26 is due to π -π* transitions. Figure(C6) compound shown λmax at 319nm Abs. 3.7 ε. 74, λmax 335 nm Abs. 3.7 ε. 74 is due to π -π* transitions, λmax 354 nm Abs 3.02 ε. 60.4 and λmax 663 nm Abs 0.258 ε. 5.16. More of these transitions are caused by unsaturation and other active groups, while the remaining peaks caused to affect the solvent toluene-ethylacetate. The compounds separated from the hot extracted oil of the A. herba-alba plant have also shown several effective groups in UV spectroscopy, which showed absorption at certain wavelengths in fig (5-2).

The resulted are figure (H1) shown λmax at 278 nm and Absorbance Abs. 3.8nm and Molar Extinction ε. 76 is due to the π-π* transitions and λmax 410 nm Abs. 0.027 ε. 0.54. Figure (H2) compound showed λmax at 403 nm Abs. 1.19 ε. 23.8 is due to n-π* and ε. 9.2, λmax 421 nm Abs. 1.27 ε. 25.4 is due to π-π* and λmax 444 nm Abs. 1.4 ε. 28. Figure(H3) compound showed (λmax at 275 nm Abs. 4 ε. 80, λmax 384 nm Abs. 0.639 ε. 12.7 is due to n-π* and λmax at 659 nm Abs. 0.258 ε. 5.1 due to π-π*.

Table (3-11) shown UV-vis spectra of *A. herba-alba* plant that isolate from cold and hot aquouse extract volatile oil of *A. herba-alba plant*

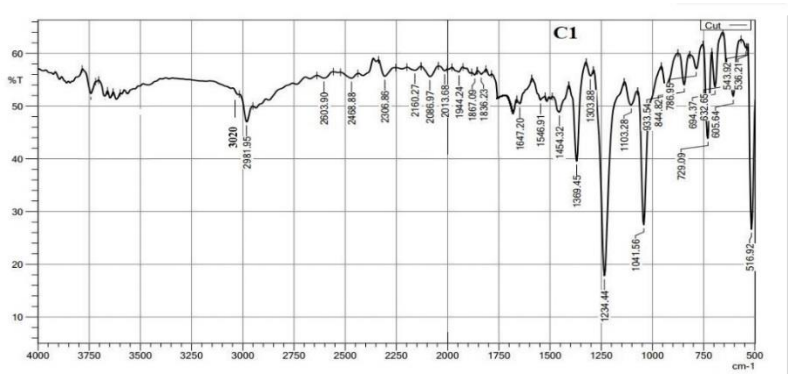
Cold Aqueous extract										
No.	**Compound**	**λ_{max} (nm)**			**Abs.**			**ε L. mol^{-1}. cm^{-1}**		
1	**C1**	278 645	390	418	3.57 0.462	1.02	0.224	71.4 9.24	20.4	4.48
2	**C2**	407 609	511 664	532	2.26 0.229	0.237 1.057	0.28	45.2 4.58	4.74 21.1	5.6
3	C3	275	406	661	4	1.115	0.476	80	22.3	9.52
4	C4	283	409	667	3.92	0.522	0.168	78.4	10.44	3.36
5	**C5**	273	695	-	4	0.063	-	80	1.26	-
6	C6	319 663	335	354	3.7 0.258	3.7	3.02	74 5.16	74	60.4
Hot Aqueous extract										
No.	**Compound**	**λ_{max} (nm)**			**Abs.**			**ε L. mol^{-1}. cm^{-1}**		
1	**H1**	278	410	-	3.8	0.027	-	76	0.54	-
2	**H2**	403	421	444	1.19	1.27	1.4	23.8	25.4	28
3	H3	275	384	659	4	0.639	0.258	80	12.7	5.1

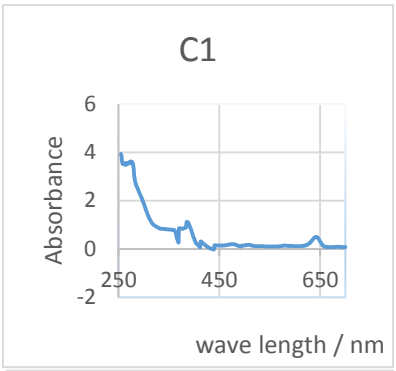
Table (3-12) Table (3-12) determine the fraction OF column chromatography by Ft-IR Spectra in cold and hot aquouse extract volatile oil of *A. herba-alba plant*

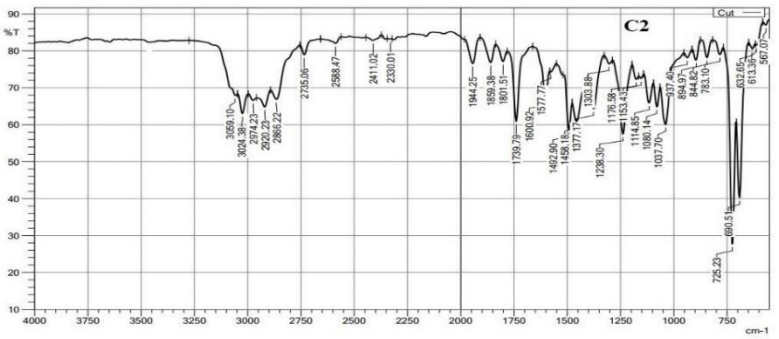
Cold Aqueous extract										
No.	**Compound**	**Assignment Cm^{-1}**								
		OH Str.	**C=C Str.**	**C.H. Str. Alk.**	**OH Bend**	**C=O Str.**	**C≡C**	C=C	**CH Bend**	**C-O**
1	**trans-aryophyllene**		-	2981	-	1735	-	1647 trans	1454	1235
2	**Beta-myrcene**		3060	2974				1601	1458	1238
3	**lineatine**		**3024**	2935		**1735**			1446 CH_3	1234 ether
4	**Ethynyl-4-methoxy-4-methyl-2-cyclobuten-1-ol**	**3260**	2974-2897	**2931**	1384-1419	1647	**2129 C≡C**		**1454**	1042 ether
5	**Caryophyllene oxide**		**3025**	**2978**		**1740 ester**		**1600 cyclic**	1455	1239

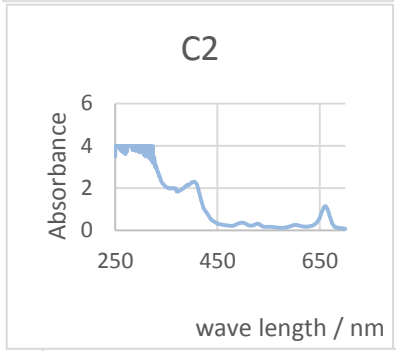
6	**camphor**		3060	2979		1736			1450	1234

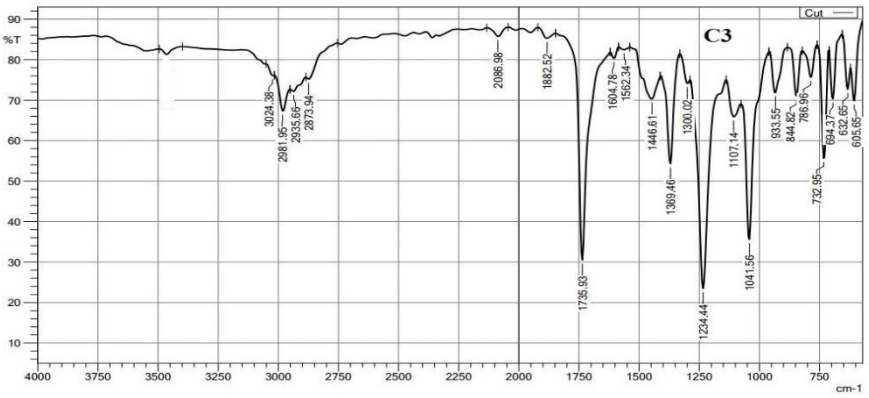
Hot Aqueous extract										
NO.	**Compound**	**Assignment Cm^{-1}**								
		NH	**OH Str.**	**C.H. Str. Aro.**	**C=C Str.**	**C.H. Str. Alk.**	**OH Bend**	**C=O Str.**	**CH Bend**	**C-O**
1	**cis-Hydroxydavanone**		**3260**		2974	2932	**1384**	1647	1450	1083
2	**vulgarin**		**3400-3600**		3002	2924	**1377**	1708	1423	1215
3	**Dihydroxanthin**	**3464**		3024	2978	2866		1735	1458	1114

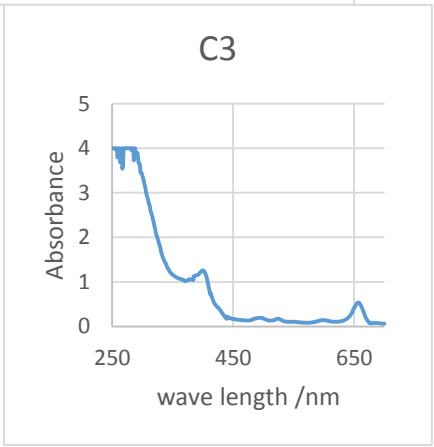












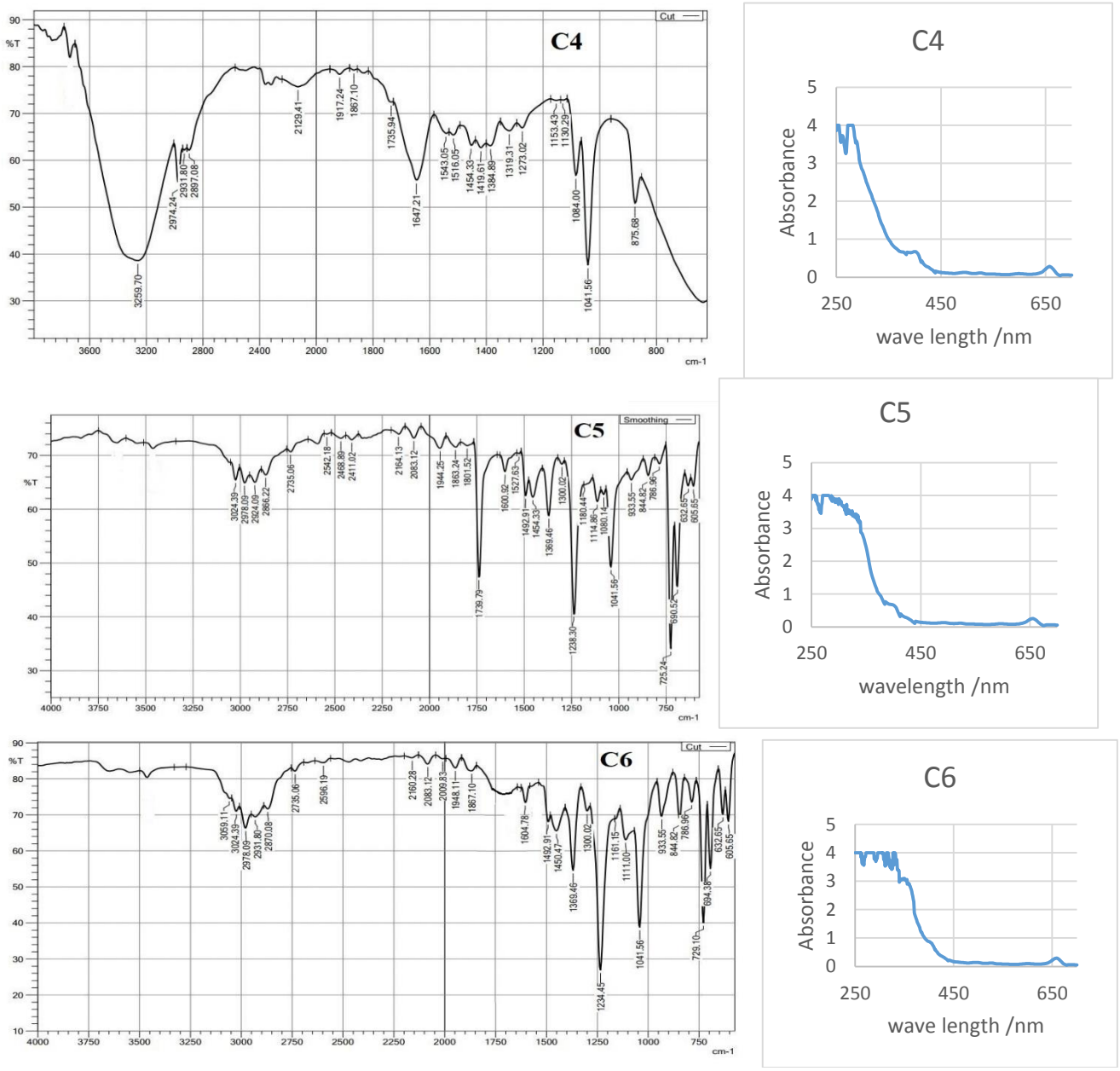
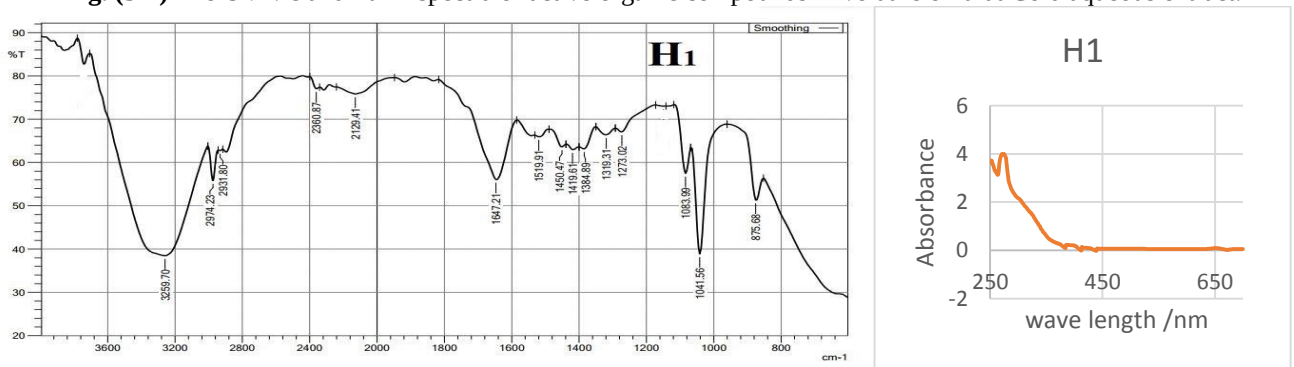


Fig. (5-1) The UV-vis and Ft-IR spectra of active organic compounds in volatile oil that Cold aqueous extract.



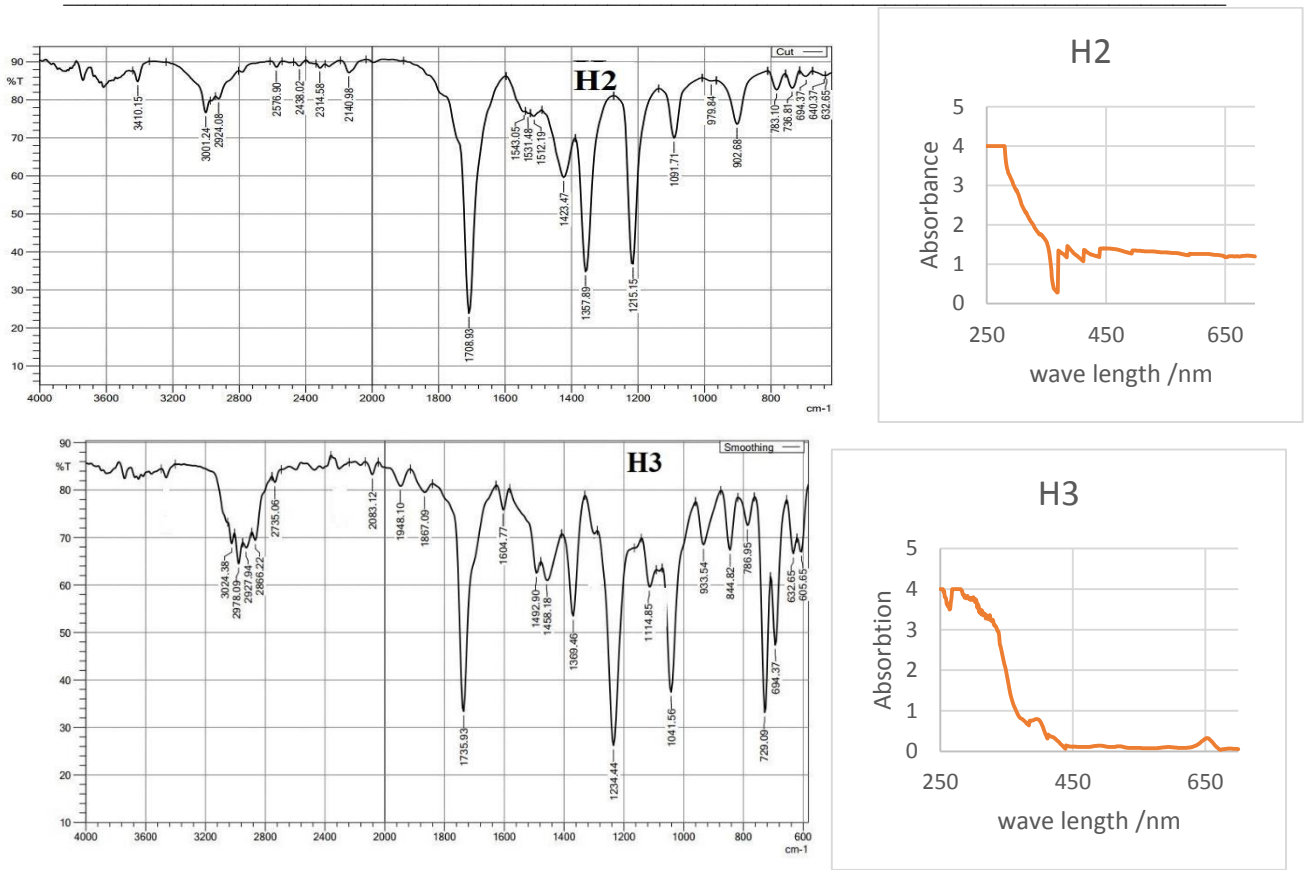


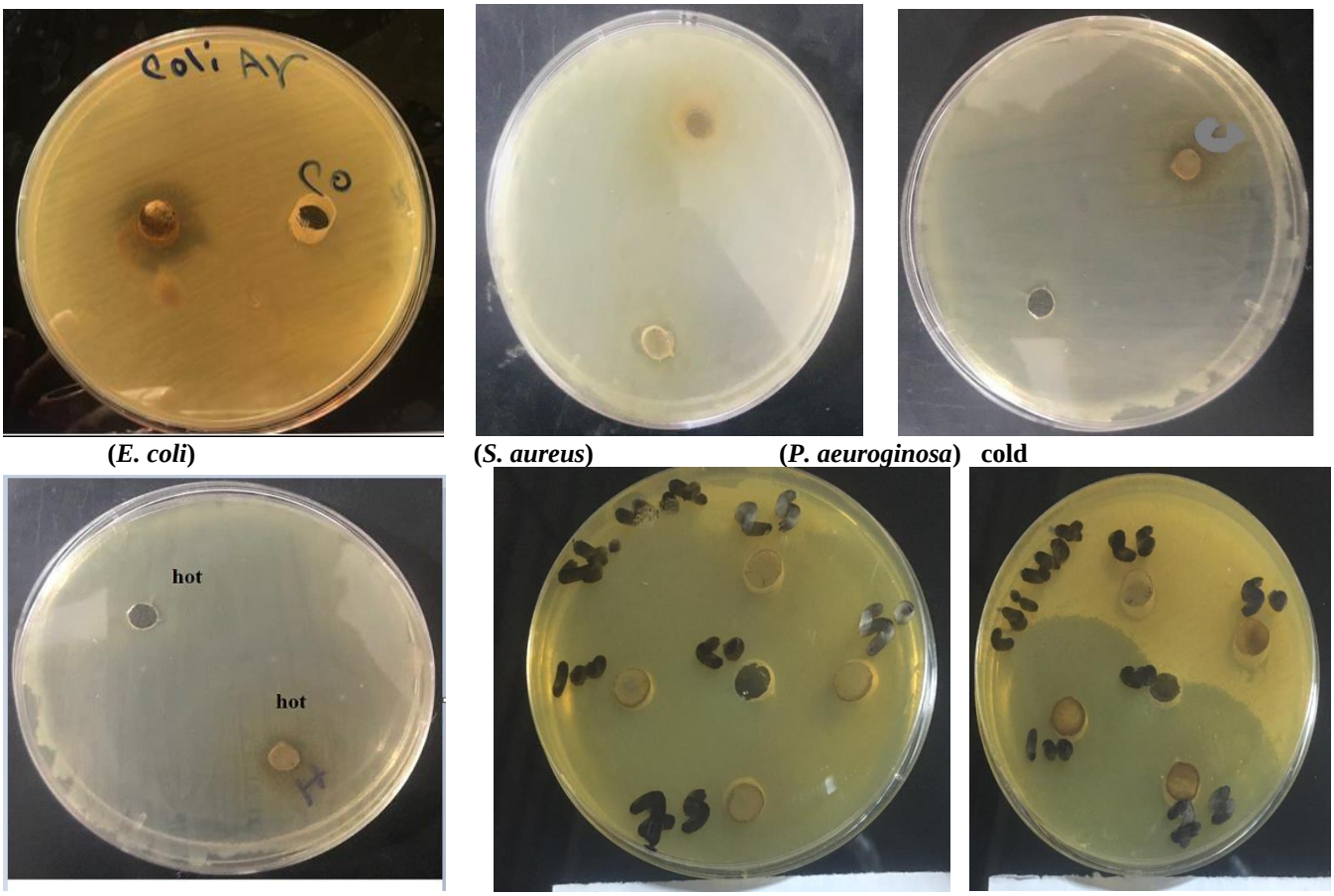
Fig. (5-2) The UV-vis and Ft-IR spectra of active organic compounds in volatile oil that Hot aqueous

3.8.1 Investigation of the Antimicrobial Activity

Antibiotics are sometimes associated with adverse effects that include hypersensitivity. Therefore, research for new antimicrobial substances exhibiting minimal side effects from natural sources is needed. Three types of selective bacteria (two Gram-negative and one Gram-positive have been used in this study for the detection of the impact of the volatile A. herba-alba plant oil on it due to the pathological effects that are caused by these microorganisms on human and animals and the feed decay [39,40] The sizes of inhibition of culture are summarized in Table (1-8), which shows volatile oils of the artemisia used. Oil is diluted in DMSO [36-37]. The disc diffusion method and the determination of the Minimal Inhibitory Concentration (MIC). The sizes of inhibition of culture summarized in Table (1-3) and fig.(4-1) shows the Effects of extracting volatile oil on the microbial activity of three types from bacteria. The study revealed the biological efficacy of the oil extract of the A. herba-alba plant in hot and cold extracts. The cold oil extract has little efficacy against Staphylococcus aureus (gram-positive)and E. coli (gram-negative). In the concentration of (25,50,75,100%) using a solvent of dimethyl sulfoxide (DMSO), the efficacy diameter was shown (0,8,10,18mm) respectively of cold extract in Staphylococcus aureus. Still, at a concentration of (25,50,75,100%), the efficacy diameter was shown (0,30,35,38mm) respectively of hot extract in Staphylococcus aureus. The liquid concerning the oil does not interfere with the agar medium with the plate. At a concentration of (25,50,75,100%) the efficacy diameter was shown (0,6,8,9mm) respectively of cold extract in Pseudomonas aeruginous. Still, at a concentration of (25,50,75,100%), the efficacy diameter was shown (0,8,10,12mm) respectively of hot extract in Pseudomonas aeruginous. At a concentration of (25,50,75,100%) the efficacy diameter was shown (0,13,14,16mm) respectively of cold extract in E. coli. Still, at a concentration of (25,50,75,100%), the efficacy diameter was shown (0,20,40,45mm) respectively of hot extract in E. coli.

Table (1-8) Effects of extracting volatile oil on the microbial activity of three types from bacteria

Oil	Con. µl	Diameter zone of Inhibition microbes (m.m)		
		Staph. Aureus	*P. aeruginous*	*E.Coli*
A. herba-alba cold Aqueous solvent	25%	0.0	0.0	0.0
	50%	8	6	13
	75%	10	8	14
	100%	18	9	16
Oil	**Con. µl**	**Diameter zone of Inhibition microbes (m.m)**		
		Staph. Aureus	*P. aeruginous*	*E.Coli*
A. herba-alba hot Aqueous solvent	25%	0.0	0.0	0.0
	50%	30	8	20
	75%	35	10	40
	100%	38	12	45



(***E. coli***) (***S. aureus***) (***P. aeuroginosa***) **cold**

(***P. aeuroginosa***) **hot** (***S. aureus***) **hot** (***E. coli***) **hot**

Figure (7-1) Effects of extracting volatile oils on the microbial activity of three types of bacteria

Conclusion

Cold and hot aqueous solvents were used to extract the aerial section of the Artemisia Herba-alba plant. Cold and hot aqueous solvent qualitative laboratory analyses of Artemisia Herba-alba plant Separate the oil from the Artemisia Herba-alba plant's extract. Gas chromatography and mass spectra were used to isolate several active chemical components from the Artemisia Herba-alba plant. Cold and hot extract oil from Artemisia Herba-alba plant were used to determine the antibacterial activity of active organic components. The results of GC-MS techniques got to show that the accompanying mixtures, camphor, filifolide A, 1-ethynyl-4-methoxy-4-methyl-2-cyclobuten-1-ol, lineatin, 2-pentanoylfuran, davana ether, caryophyllene oxide, beta-Myrcene, vulgarin, trans-caryophyllene, And bis(2-ethylhexyl)phthalate are available in A. herba-alba oil that extricated by cold aqueous dissolvable. Acquired demonstrate that the accompanying mixtures, 2-methyl-4-nitroresorcinol, cis-hydroxydavanone, vulgarin, and

dihydroxanthin, are available in the simultaneous are available in Artemisia oil that extracted by hot aqueous solvent. The primary goal of this research is to determine the contents of the volatile oil of the Artemisia plant Herba-alba using the mass spectrometry separation technique and knowledge of the plant's qualitative diagnostic and molar mass. The biological efficiency of A. herba-alba oil extracted in hot and cold aqueous media on bacteria was investigated and a comparison between them.

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