A Separation and Identification of Some Volatile oils and Producing Compounds from Column Chromatography from The Iraqi Cuminum Cyminum L. Seeds and Antibacterial Effect

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Abstract
Phytochemical screening of some active components from the seeds of Iraqi flora plants under study, Cuminum Cyminum L. Where was used a continuous extractor apparatus (soxhlet) by using different organic solvents which are [Pet. ether (Cu1), Chloroform (cu2), Ethyl acetate (Cu3), Ethanol (Cu4) and hot water (Cu5). Our current study was confirmed that the separation and identification of many compounds of volatile oil for the seeds of Iraqi cumin plant by using converted distillation device (Clevenger) for light oil and identify these compounds by using (GLC) technique and they are as following (∝-Pinene, Menthol, Terpine, Limonene and Linalool). The highest concentration was representen in Linalool (33.5%) and the lowest concentration was represent in Limonene (1.58%). Also, Phenolic compounds were separated and identified by using column chromatography (CC) technique, and we obtained three fractions (F1, F2 and F3). Five Phenolic compounds were identified by using (HPLC) technique in the fraction (F2) and they are as following (Caffeic acid, Syringic acid, P-Coumaric acid, Apigenin and Rutin), while four Phenolic compounds were appeared in the fraction (F3) and they are (Syringic acid, P-Coumaric acid, Kaempferol and Quercetin) and it has observed that Caffeic acid was appeared only by using column chromatography (CC) technique. Also, Rutin appeared at the highest concentration from the rest compounds in the fraction (F2). In order to get the benefit from the identification of some active compounds, from the seeds of Iraqi Cumin plant, the study included their effect on two types of bacteria that are pathogenic to humans (Escherichia coli and Staphylococcus aureus) through our observation to the inhibitory that occured in the dishes. The highest inhibition was (32.33 mm) that caused by hot water extract (Cu5) at the concentration of 100% and the lowest inhibition was (15.25 mm) that caused by the hot water extract (Cu5) at concentration 25% in bacteria (G+) Staphylococcus aureus, while the highest inhibition in bacteria (G-) Escherichia coli was (26.17 mm) that cause by hot water extract (Cu5) at concentration 75%, and the lowest inhibition was(13.63 mm) caused by Ethanolic extract (Cu4) at concentration 75%.

Keywords: Cuminum Cyminum, Volatile oils, Column Chromatography, antibacterial

1. Introduction

Spices are bio-nutrient supplements that enhance the taste, flavor and aroma of food and also treat several diseases. Cumin (*Cuminum cyminum*) is one such most popular spice that is used as a culinary spice for their special aromatic effect. The proximate analysis of the cumin seeds reveals that they contain fixed oil, volatile oils, acids, essential oils, protein and other elements. In cumin, contains an important component such as pinene, cymene, terpinene, cuminaldehyde, oleoresin, thymol and others that have shown their uses according to the disease and it has proved several benefits with the help of availability of nutrients.It is an important element of iron for energy, immunity systems, lactation and skin diseases.(Singh *et al* ., 2017)

(*Cuminum cyminum* L.) is a member of the Apiaceae family that originated in the Mediterranean region, Turkistan, and Egypt, but has spread to various arid and semi-arid regions in the world, including the Middle East, India, and Turkey. It is one of the oldest and economically important plant species. It is drought-tolerant and mostly grown in Mediterranean climates. The plant is grown from seed, sown in spring, and needs fertile and well-drained soil. The fruit has a 4–5-mm-long lateral fusiform or ovoid achene, containing a single seed (Mnif and Aifa, 2015).

The most important chemical component of cumin fruits is essential oil content, ranging from 2.5% to 4.5%. The ripe seeds of cumin are used for essential oil production, the essential oil is responsible for the characteristic cumin odor. This odor and flavor is due principally to the aldehydes present. Studies of the chemical composition of cumin oil from different countries showed the presence of the following components: α-pinene, Myrcene, limonene, 1-8-cineole, p-menth-3-en-7-ol, pmentha-1, 3-dien-7-ol, caryophyllene, β-bisabolene, β-pinene, P-cymene, β-phellandrene, D-terpinene, cuminic aldehyde, cuminyl alcohol, β-farnesene together with much smaller quantities of α- phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral, and other studies show that cumin essential oil mainly

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contains monoterpene aldehydes. The major compounds include cumin aldehyde (p-isopropylbenzaldehyde), terpinene, α- and β-pinene, ρ-cymene, ρ-mentha-1,3-dien-7-al, cuminyl alcohol and β-farnesene (Nadeem and Riaz,2012)

Cumin seeds are well reported for their antioxidant, antibacterial,insect-killing potential, antiinflammatory, pain-relieving, antitumor, antiallergic, tension-preventive, antihyperlipidemic, bronchodilatory, immunomodulatory anti-osteoporotic, antifungal, these excellent pharmacological potentials of cumin are mainly attributed to phenols, terpenoids, and flavonoids (Singh et al, 2021) .

Escherichia coli also known as E. coli, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents (Tenaillon et al,2010)

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen, Although S. aureus usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning (Tong et al.,2015).

Taxonomical classification

Biological name	Cuminum cyminum L
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaseae
Genus	Cuminum
Species	Cuminum cyminum
(Prajapati et al.,2019)	

Biological name	Staphylococcus aureus
Kingdom	Bacteria
phylum	Firmicutes
Class	Bacilli
Order	Bacillale
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*Staphylococcus aureus*
(Jawetz et al., 2004)	

Biological name	Escherichia coli
Kingdom	Bacteria
phylum	Proteobacteria
Class	Gammaprotobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	Escherichia
Species	Escherichia coli
(John et al., 2020)	

2. Materials and Method

1- Collection of seeds

The seeds of Cuminum cyminum L. were collected from Mosul Dam area in May 2021 and they were classified in the Directorate of Medicinal Plants Development Project in Mosul Dar area, which is affiliated to the Ministry of Agriculture and Agricultural Reform. Then, the seeds were cleaned from the dust and then put in paper batch and kept in a place away from moisture until using them.

2- Preparation of some plant extracts using continuous Soxhlet device:

The seeds cuminum cyminum L. were crushed by electric mill, where 25 g of it. The well-ground powder was placed in the Soxhlet batch system and 200 ml of petroleum ether was added to the flax seeds extracted oil. The extraction process continued at the rate 7 hours per day until the end used in the device became colorless. In the end, the extract was concentrated by using a rotary vacuum evaporator (RVE) (Harborne, 1984).

Four solvents were used in the soxhlet device by the sequence solvent system concept: pet-ether (40-60) C° (CU1), chloroform (CU2) Ethyl acetate (CU3) and Ethanol (CU4). Hot aqueous extract (CU5) was employed using Grand method (Ale Grand, 1998).

3- Volatile oils extracted by converted Clevenger Apparatus

In this process the volatile oil compounds was extracted from the seeds of the study plant by using celvenger device to extract the light oil and connected with a volumetric flask with a capacity of 500ml and used 15 gm of the seeds of Cuminum cyminum L. as powder was mixed with 200 ml of (D.W) and then the distillation process was carried out with the boiling point 100°c and the process of distillation lasted between (1– 2) hrs . The distilled water was put in separating funnel (100ml) and 50ml of ether was added to it for two stages, shaking the mixture well and then left to settle, two layer and concentrated it by using rotary vacuum evaporator. The crude oil was placed in the bottle and kept in the refrigerator until it used and identified.
(Britich, 1958).

4- Chromatographic determination of volatile oils using GLC- analysis from the seeds of Cuminum cyminum L

Chromatographic analysis of the diagrams were investigated in which the retention time of each volatile oil compound was determined for study sample that compaired to the authentic sample retention time.

The separated volatile oil compounds were identified in the laboratories of the ministry of Science and Technology / Dept. of Environoment and water by GLC model Shimadzo, Japanese,2010 using ionized flame detector and using the injection area and the detector (280 and 330 °c) while the

column tempera-ture starts from (120- 280 °c) at rate of 8°c / min. using passive nitrogen gas as a carrier gas at a rate of 100 kp.

5- Phenolic Compounds that separated by Column Chromatography

After preparing the Chromatographic Column and filling it with silica gel of type(60 mesh) and saturated with petroleum ether (40-60)0C, It was taken (5 ml) from the extract of ethanol CU4 of the seeds of Cuminum cyminum L. and pass from the top of Column Chromatography, then following solvents were used as mobile phase and according to the polar system using petroleum ether and the following using Chloroform only and then using Ethyl acetate alone, after that we used Ethyl acetate:Ethanol at ratio 1:1 volume / volume, and then Ethanol was added alone, finally Methanol was added, three fractions (F1,F2,F3)were obtained and kept in the refrigerator until Identificated by Chromatographic methods .

6- Identification of the phenolic compounds by using HPLC-UV device

The identification of the phenolic compounds was conducted in the laboratories of the Ministry of Science and Technology / Dept. of Environment and Water Resources, by using high performance liquid chromatography device (HPLC) type Sykamn of German origin with a flow rate of 1.3 (ml.min-1). The mobile phase is (A), which includes (methanol: D-W: formic acid, (70:25:5) with the column (18-ODS) has dimensions (25 cm*4.6 mm) and the responses were detected at the UV-280 nm wavelength.

7-Sensitivity test method (diffusion by pits)

The antimicrobial susceptibility test was done using well diffusion method according to (25,50,75,100). Mueller-Hinton agar was prepared by adding 39 g of Muller-Hinton agar to 1 Liter of distilled water then mixed very well with heating using hot plate stirrer until boiling. After that, the agar was autoclaved at 115 °C for 15 min using portable autoclave. The agar was cooled and poured into sterile petri dish and left to solidify. After that, 6 mm diameter wells was made in the agar plates. Fresh cultures were prepared of Staphylococcus aureus and Escherichia coli and adjusted to 0.5 McFarland standard (1.5 x 108 CFU/ml). The agar plates was inoculated with specific bacteria suspension using sterile cotton swab immersed in the bacterial suspension and spread on the surface of Mueller-Hinton agar and left for (15) minutes. After that, and 0.1 ml of the extract with different concentrations dissolved with (DMSO as a solvent) was loaded in the corresponding wells, and standard antibiotic disc (Streptomycin, Levofloxacin) was used as control positive while 0.1 ml of DMSO was used as control negative. The plates were incubated for 37 °C for 18 h, and the inhibition zone was measured using digital caliper (perez et al.,1990).

3. Results and Discussions

1-Chromatographic determination of volatile oil compounds by GLC analysis for the seeds of cuminum cyminum L:

Chromatographic analysis of the charts were obtained in which the retention time of each compound was conducted for the study samples compared to the standard sample retention of Alpha – pinene (6.403 min.), Menthol (9.790 min.), Terpinen (4.336 min.), Limonene (7.356 min.), Linalool (3.553 min.). Also, the concentration of these identified volatile compounds were carried out by using the percentage ratio. The highest concentration was found in Linalool compound and the lowest concentration was found in Limonene compound. And cuminaldehyde was identified by GLC technique, it was appeared at concentration (33.1) % and the rotation time at (5.887) min. table (1), figs (1, 2, 3, 4, 5, 6, 7)

Table (1) The concentration (%) of volatile oil compounds by using GLC technique of normal aqueous extract from the seeds of cuminum cyminum (L)

No	Compounds	Rt(min.)	Normal aq. extract (%)
1	Alpha-pinene	6.403	5.8
2	Menthol	9.790	12.5
3	Terpinen	4.336	24.5
4	Limonene	7.356	1.58
5	Linalool	3.553	33.5

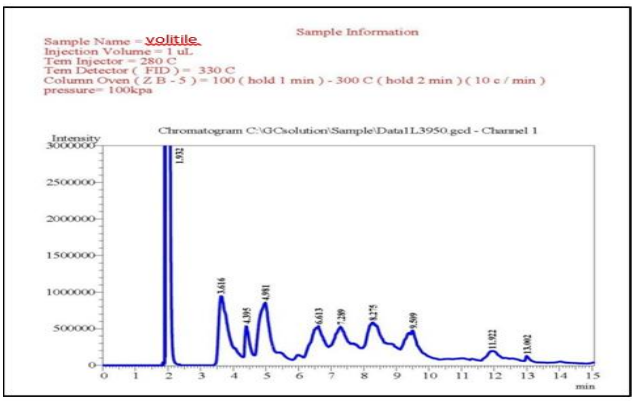


Fig (1): The volatite oil compounds by the Clevenger apparatus

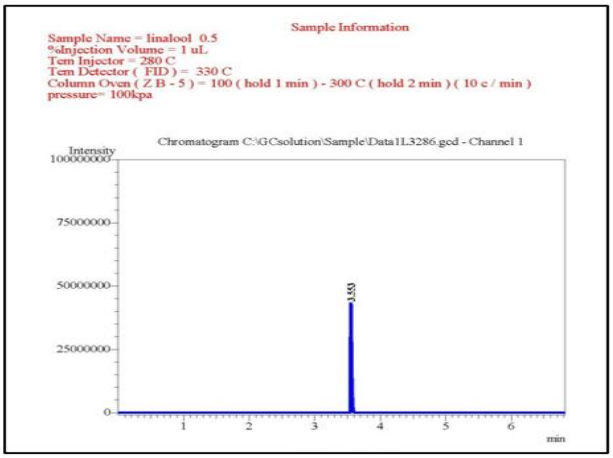


Fig (2): The standard curve of linalool

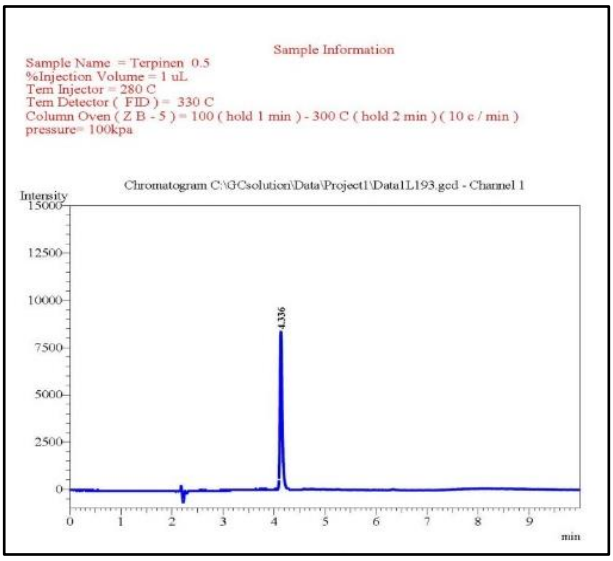
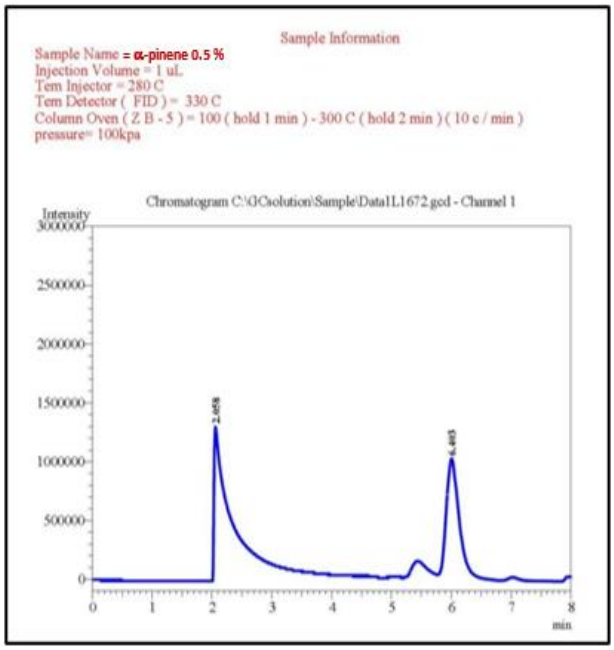


Fig (3): The standard curve of Terpinen



Fi g (4): The standard curve of α-pinene

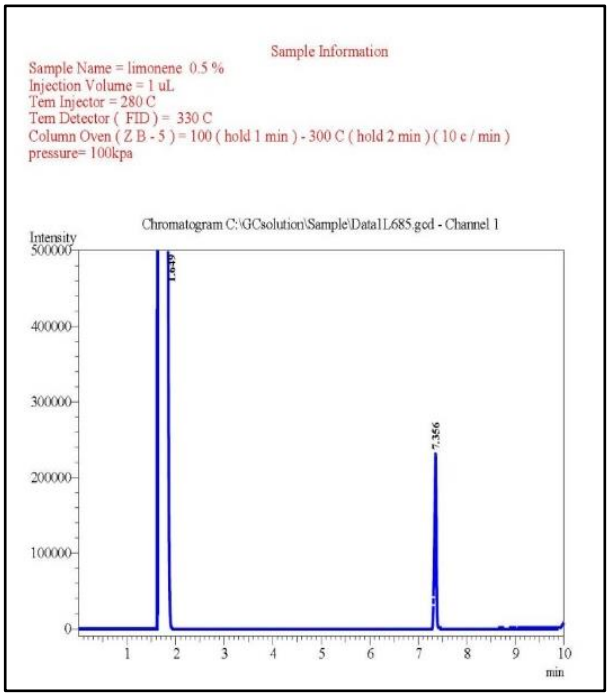


Fig (5): The standard curve of limonene

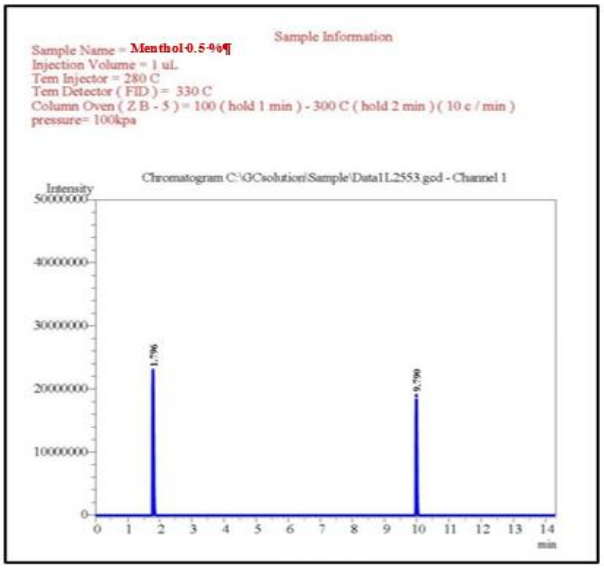


Fig (6): The standard curve of Menthol

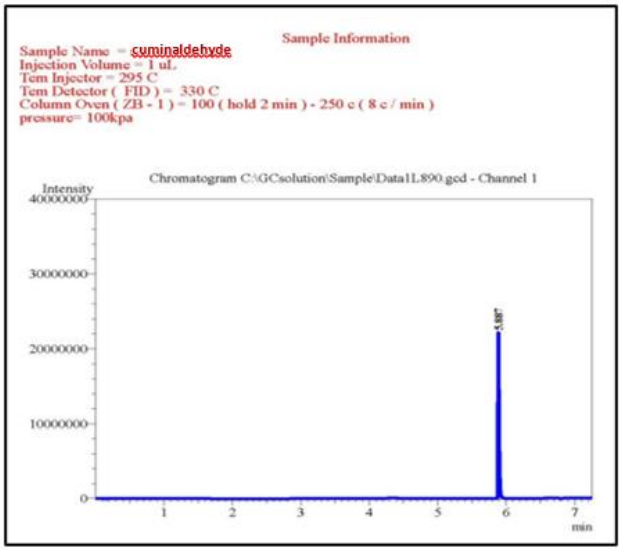


Fig (7): The standard curve of Cuminaldehyde

2- Identification of number of phenolic compounds that separated by Column Chromatography and identified by using HPLC technique for the seeds of *cuminum cyminum* L

The identification by using HPLC was showed that the separation by Column Chromatography is characterized by the appearance of compounds in the form of rings, and the chart of analysis was showed that the retention time of each sample was obtained and compared with standard. The retention time for Caffeic acid was (2.453 min), Syringic acid (3.28min), P-couramic acid (4.44 min), Apignin was (6.60 min), Rutin (8.00 min), Kaempferol (7.75 min) and Quercetin (11.02 min). This indicates the presence of the phenolic compounds in the seeds of cuminum cyminum L Caffeic acid was showed in F2 (Ethanol solvent),the concentration of Caffeic acid was (0.095896 mg/g) and the rotation time (2.55 min), Syringic acid was also detected in F2 at (3.21) and the concentrations (0.079584 mg/g), and it was presented in F3 (Methanol) at (3.10 min) and the concentration (0.113176 mg/g), but P-couramic acid

was presented with concentration (0.09004 mg/g) and the rotation time (4.58min) in F2, and also appeared in F3 at (4.50 min) and the concentration (0.04892 mg/g).

While Apignin was presented at (6.71 min.) and with concentration of (0.016344 mg/g) in F2, and Kaempferol was only presented in F3 with the concentration (0.028552 mg/g) and the rotation time (7.82 min).

Whereas Rutin was only presented in F2 with a rotation time (8.02) and a concentration of (0.138824 mg/g). Finally, Quercetin (11.00 min) was detected in F3 at the concentration (0.018824 mg/g). table (2), figs (8,9, 10,11,12,13,14,15).

Table (2): The concentration of isolated phenolic compounds from column chromatography and identified by using HPLC technique from Iraqi seeds of *cuminum cyminum* L. plant

No	Standard Phenolic Compounds	Standard of Retention Time (min)	F2		F3	
			The Concentration mg/g	Retention time (min)	The Concentration mg/g	Retention time (min)
1	Caffeic acid	2.453	0.095896	2.55	----	----
2	Syringic acid	3.28	0.079584	3.21	0.113176	3.10
3	P-coumaric acid	4.44	0.09004	4.58	0.04892	4.50
4	Apigenin	6.60	0.016344	6.71	----	----
5	Rutin	8.00	0.138824	8.02	----	----
6	Kaempferol	7.75	----	----	0.028552	7.82
7	Quercetin	11.02	----	----	0.018824	11.00

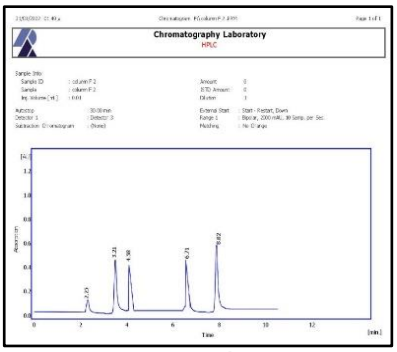


Fig (8): The standard curve of the separated phenolic compounds by cc that identified by HPLC

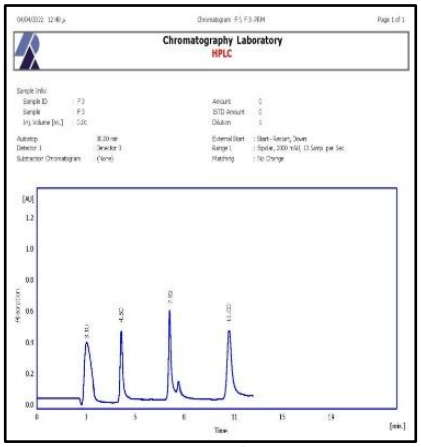
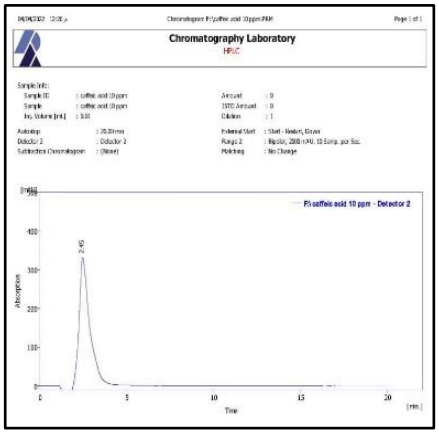


Fig (9): The standard curve of the separated phenolic compounds by cc that identified by HPLC



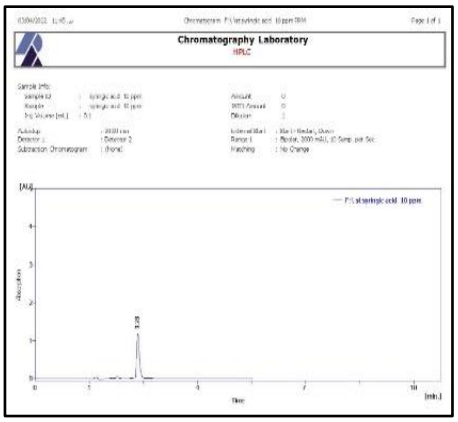


Fig (10): The standard curve of caffeic acid

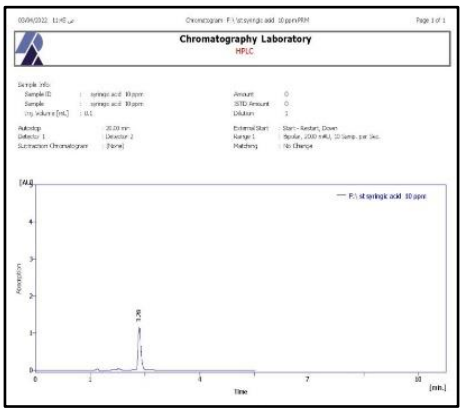


Fig (11): The standard curve of syringic acid

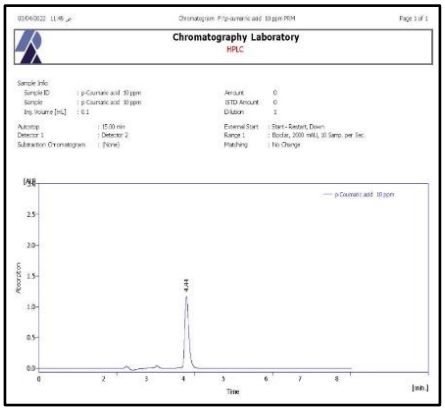


Fig (12): The standard curve of p-coumaric acid

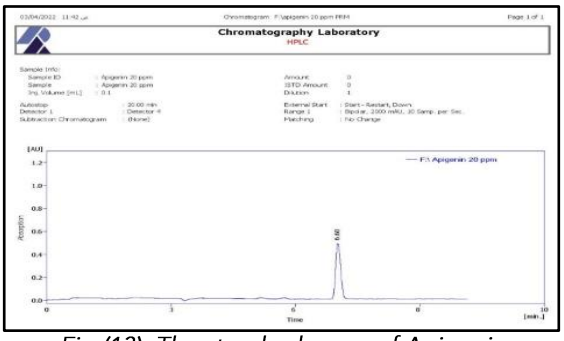


Fig (13): The standard curve of Apigenin

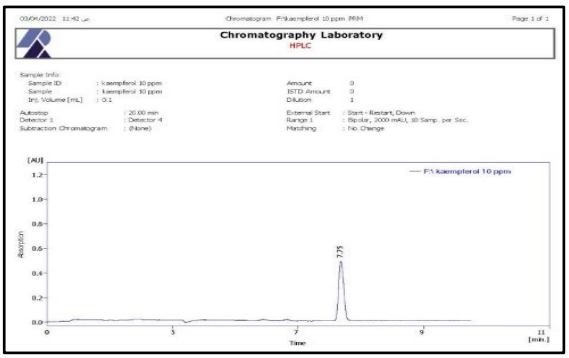


Fig (14): The standard curve of Kaempferol

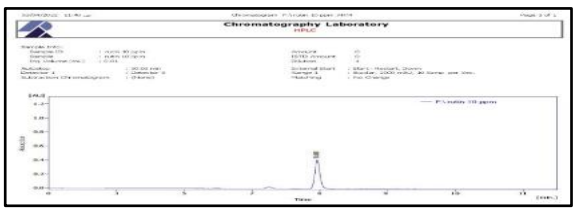


Fig (15): The standard curve of rutin

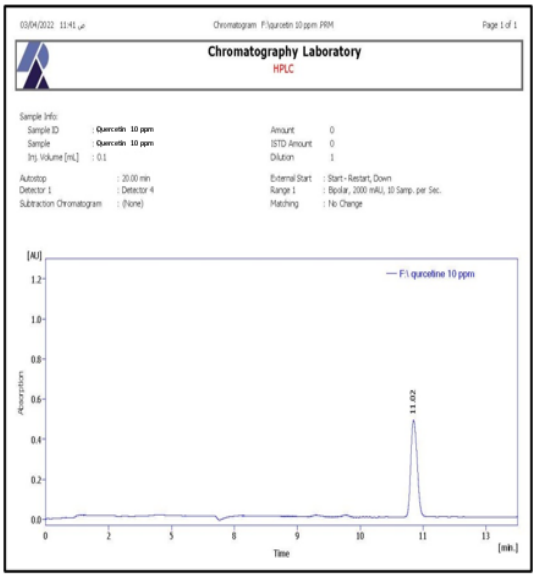


Fig (16): The standard curve of quercetin

2-Effect the active compounds for the seeds of *cuminum cyminum* L. in *Staphylococcus aureus* and *Escherichia coli* by diffusion method

The inhibitory activity was tested for some the plant extracts (Ethanol, Hot aqueous) and by four concentrations (25,50,75,100) % for two types of the using bacteria in the study to know the inhibitory effect of each type from the bacteria,and each concentration from the different extracts, table(3), fig (17).

Table (3): The effect of concentration of some phenolic extracts from seeds of Iraqi *cuminum cyminum* L. on two types of Bacteria (*Staphylococcus aureus, Escherichia coli*)

Type of Extract	Concentration	*Staphylococcus aureus*	**yperus rotundus L. on two type tion of some phenolic extracts.from tubers of Iraqi Cyperus rotundus L** 24362436243624362436243624362436243624362436243624362436 *Es*24362436243624362436243624362436243624362436243624362436 *cherichia coli*
Ethanol	100%	31.6	17.7
	75%	26.8	13.6
	50%	19.1	13.9
	25%	0.0	0.0
Hot water	100%	32.3	24.8
	75%	30.8	26.1
	50%	24.1	21.2
	25%	15.2	0.0

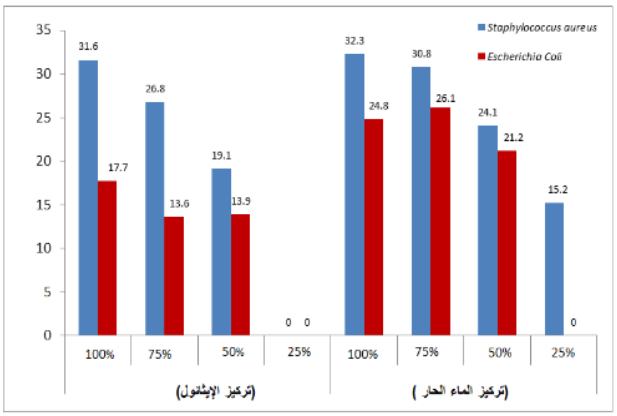
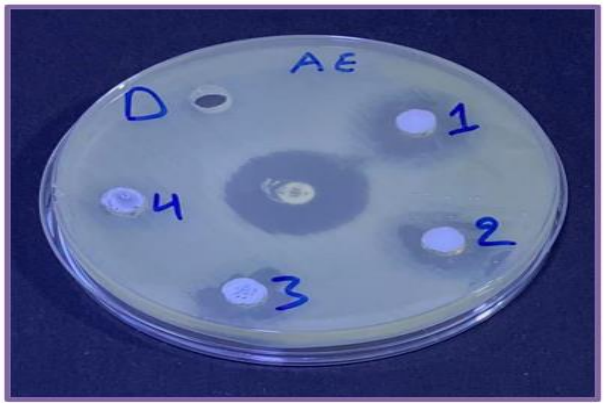


Fig (17): The effect of the plant (Ethanol, Hot water) on the two types of bacteria (Staphy) ococcus aureus, Escherichia coli)

The results was showed in table (3) that the extract of ethanol was been a inhibitory effect toward two types from the using bacteria in the study, at the concentration(100%) the inhibitory effect was been high, the inhibitory diameters was appeared for two types Staphylococcus aureus and Escherichia coli at (31.6, 17.7) mm respectively, and the concentration (75%) the inhibitory effect of each type from the bacteria at (26.8,13.6)mm respectively,while the concentration (50%) was recorded the inhibitory effect in Staphylococcus aureus and Escherichia coli (19.1,13.9) mm respectively. Whereas the

concentration (25%) the extract of ethanol was not appeared any inhibitory effect for the both types from the bacteria, picture (1) and (2).



Pic (2) The effect of Ethanol extract on Staph.aureus (G^+).

A :Ethanol

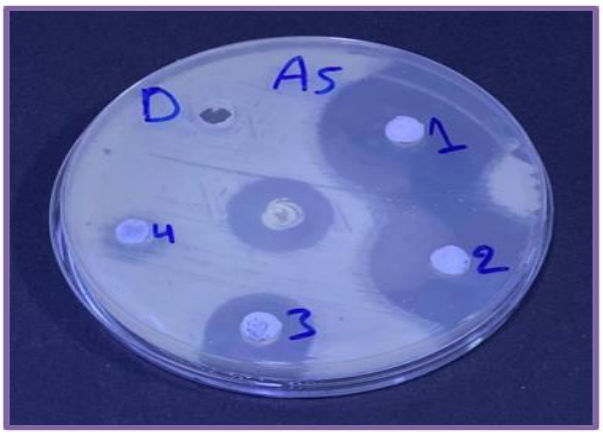
S :Staph.aureus

1: Con. 100%

2: Con. 75%

3: Con. 50%

4: Con. 25%



Pic (1) The effect of Ethanol extract on E.Coli (G^-).

A :Ethanol

E :E.Coli

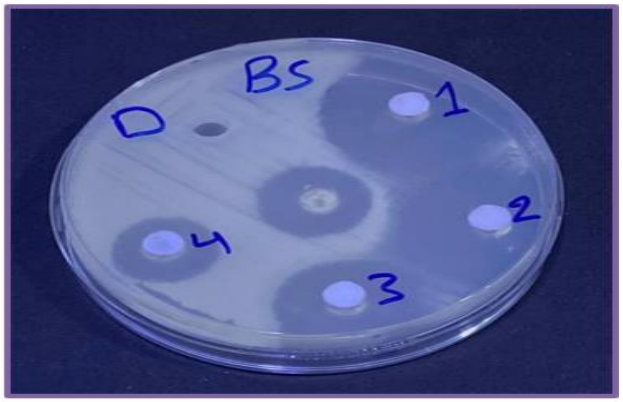
1: Con. 100%

2: Con. 75%

3: Con. 50%

4: Con. 25%

And also the hot aqueous extract was appeared very high of the inhibitory effect for the different types of bacteria, table (3), at the concentration (100%) the inhibitory amount for *Staphylococcus aureus* and *Escherichia coli* (32.3,24.8)mm respectively, and the concentration at (75%) the inhibitory diameters for *Staphylococcus aureus* and *Escherichia coli* was (30.8,26.1) mm, while the concentration at(50%) was recorded the different inhibitory diameters with amount (24.1, 21.2) mm respectively, and the concentration at (25%) was showed the inhibitory effect for *Staphylococcus aureus* at amount(15.2mm) while it was not recorded any the inhibitory effect for *Escherichia coli* at the same concentration .picture (3) and (4).



Pic (4) The effect of Hot water extract on Staph.aureus (G^+).

B :Hot water

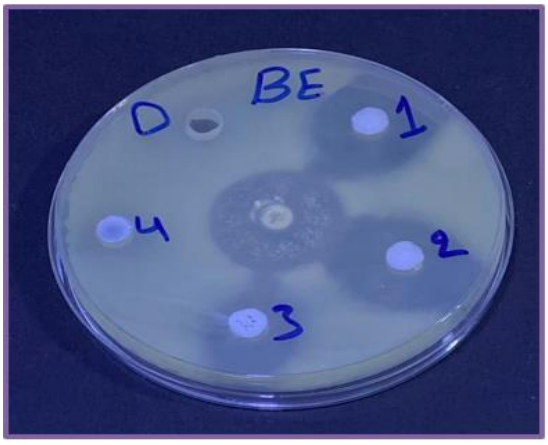
S :Staph.aureus

1: Con. 100%

2: Con. 75%

3: Con. 50%

4: Con. 25%



Pic (3) The effect of Hot water extract on E.Coli (G-).

B :Hot water

E :E.Coli

1: Con. 100%

2: Con. 75%

3: Con. 50%

4: Con. 25%

That it showed the inhibitory of the extracts of cuminum cyminum
had the high effect for resistant the bacteria compared with antibiotic

4. Conclusion

The volatile oils and phenolic compound were showed in different extracts from the seeds of cuminum cyminum (L.) which is growing in Iraq and

investigated by using chromatographic analysis with GLC and HPLC techniques.

All compounds that were showed in the various extracts of cuminum cyminum (L.) were important for multiple cases especially in the medical treatment.

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