Characterization and detection of some active compounds in seeds oil of Cumin (*Cuminum cyminum*) by GC-MS and GLC

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Abstract

The current study investigated some activities of chemical compounds which separated from *Cuminum cyminum* seeds and tested for their antibacterial activity against three types of bacteria that are pathogenic to humans and plants: *Staphlococcus aureus*, *Escherichia.coli*, *Agrobicterum rhizogenes*. The fatty acids were obtained after the soaponification process from the Petroleum ether extracted cumin seeds were separated by Soxhleet and diagnosed using (GC-MS) The volatile oils produced from cumin seeds were also separated using a Clevenger axis steam distillation device. Results, revealed that, cumin seeds are rich in with fatty acids, and this was indicated by the diagnostic results using GC-MS technology, as eight fatty acids were separated from the petroleum ether extract of cumin, including Octadecadienoic acid. Hexadecanoic acid, while the results of (GLC) showed the presence of the Cuminaldehyde in the separated oil, and that all of these compounds showed a high inhibitory effect against the bacteria used under study using the inhibitory activity test (susceptibility test) by drilling and estimating their inhibitory concentrations compared to the antibiotics used under study.

Keywords: Cumin, GCMS, GLC, Fatty acids, Cuminaldehyde.

Introduction

Medicinal and aromatic plants are characterized by containing natural chemical compounds and are known as active compounds resulting from secondary metabolic processes within the plant and are used as defense materials against pathogenic germs or microorganisms as well as for the permanence of their life [1,2].

Cuminum cyminum, belongs to the family Apiaceae. It is a highly branched annual herbaceous plant with a cylindrical stem that may reach about 30 cm in length, branched at the base. Its leaves are threadlike or finely lobed, feathery with a short neck, and its small flowers are tent-like inflorescences. The color is white, purple or pink, its fruits are brown in the form of aromatic capsules, have a distinctive, strong smell and a pungent taste. Its roots are long, cylindrical, and deep. The seeds are rectangular in shape with longitudinal stripes, which are the most widely used parts and are yellowish-brown in color [3] Medicinal plants have entered into many

fields, the most important of which is the treatment of many diseases. Attention has recently drawn on studying the effective of extracting componds from medicinal plants and using them as anti-therapeutic drugs safely and without side effects, unlike manufactured chemical compounds. The cumin plant contains many major fatty acids such as thymoquinone, acidosis. Linoleic, dithymoquinone, oleic, cortisone and palmitic cumin oil which is known to be an excellent food source of thymoquinone and cumin aldehyde and is used as a natural antioxidant [4].

Materials and Methods

The materials and methods of the current study included two axes, separating and diagnosis the active compounds from cumin seeds, and detection of their antiviral activity against pathogenic bacteria.

Active Compound Separation Hub

Cuminum cyminum was collected and classified from the markets of Mosul city from reliable sources and classified by taxonomists.

Methods for preparing plant extracts

Soxhlet was used for plant extraction based on [5]. The plant extracts were prepared using a continuous extraction device based on the researcher's method and the separation was done on the basis of the successive solvent system, as the extraction process was carried out within three different solvents, namely petroleum ether, ethyl acetate: and methanol at different temperatures according to the boiling point, 100 gm of cumin seed powder was taken after grinding and then placed in a continuous extraction device with 500 ml of each solvent, and the extraction process continued at a rate of 8 per day until the solvent used turned colorless. Rotary evaporator device RVE at a temperature of 40°C and then store the plant extracts in the refrigerator after being placed in sterile, tightly closed and opaque bottles until use[6]

Extraction of volatile oil from cumin seeds by means of a Cliffanger axis steam distillation

The volatile oil was extracted from cumin seeds using a cliffhanger connected to a volumetric flask with a capacity of 500 ml. 60 gm of cumin seed powder was mixed with 400 ml of distilled water. Then, the distillation process was carried out using a mantel at a boiling point of 100°C, and this process continued. Between (1.5-2.5) hours, after which distilled water containing volatile oil is collected and placed in a separating funnel 100 ml of it and 50 ml of ether, the mixture is shaken and left to settle, so that the upper layer is ether with oil, while the lower layer is water, the lower layer is neglected The top layer containing the volital oil is taken and the ether is evaporated using a rotary vacuum evaporator under vibratory pressure at a temperature of 25-30°C, the crude oil is kept in sterilized, opaque vial that are sealed in

a refrigerator at a temperature of 4°C until the diagnosis is made as shown in the picture (2-3) [7], [8].

Separation of fatty acids

Soapsaponification process

Crude petroleum ether extract was taken in an amount of 10 g and 100 ml of (7.5 M KOH) solution was added to it, then rellex was made to it at a temperature of 100°C for 90 minutes, and 100 ml of distilled water was added to it after cooling the mixture to room temperature to be in the form of an emulsion, and it was added to it (2×25) ml to remove the unsaponified fat for three times after placing the solution in the separating funnel, and the saponified layer was acidified by 20% concentrated sulfuric acid H_2SO_4 until the pH reached 2=(pH) and the saponified fat was taken and extracted by adding ether 25 ml depending on Separation funnel, then the upper layer was taken ether and the lower aqueous layer was neglected [9] and kept inside sterilized and opaque glass bottles in the refrigerator until it was diagnosed by GC-MS technology [10].

Gas Chromatography-Mass Spectrometer (GC-MS)

The fatty acids and terpenes of petroleum ether extract from cumin were diagnosed in Food Research Laboratories and Consumer Protection-University of Basra by a gas chromatograph connected to a mass spectrometer type GC MS QP210 Ultra from SHIMADZU of Japanese origin and contains a capillary column type methyl polysiloxane (5% Phenyl, 95) % DB-MS 5 and its dimensions (30 meters in length and 0.23 in diameter, and the thickness of the static phase is 0.25 µm) is the static phase The mobile phase is a highly purified carrier helium gas. The separation process was carried out according to the thermal system of the MS-GC device at a temperature of 40°C for one minute, then it begins to increase to 150 °C for a minute and at a rate of 5°C per minute and reaches 280°C at a rate of 5°C per minute and the temperature is stable at 280°C. for a minute. The auto-injection process was performed by injecting 1 microliter into a Gas Chromatography/Mass spectroscopy device from the upper layer containing fatty acids after the esterification process of the oil of the samples under study, type AOC-AHIMADZU, 20i+5. The separation conditions were as follows: After obtaining the results, they are processed with the GC-MS solutions program and look at the separated Peaks charts based on the spectra database of the NSTA 08 library [11].

Diagnostics of volatile oils using gas liquid chromatography (GlC)

The volatile oils produced from the cumin seed powder under study were diagnosed in the laboratory of the Ministry of Science and Technology / Department of Environment and Water. Japanese-origin 2010 model from Shimadzu company, using a flame ionized detector FID with a capillary separation column type (DM-5MS) with a diameter of 0.25 mm and a length of 30 m. As for the injection and detector area,

it was at a temperature of 295°C, and for the separation column, the temperature starts gradually from (295-300)°C and rises at a rate of 10°/min in the presence of biologically inert nitrogen as a carrier gas at a rate of 100 KP. According to the method [12].

The bacteria used in the study

Three bacterial isolates were used in this study, which were *Staph.aureus*, *E.coli*, *Agro.rhizogenes*. Diagnosed and isolated isolates were obtained from the Department of Biology, Bacterial Bank, University of Mosul/College of Science.

Equipped agricultural circles

Prepare the nutrient agar medium according to the manufacturer's instructions which are attached to the package, sterilize and cool the medium to 45°C, pour into sterilized Petri dishes and leave to cool and store at 4°C until.

Antibiotic

The sensitivity of bacterial isolates to six types of antibiotics was tested according to the standard Kirby-Bauer method and as stated in [13] and as follows tabel (1):-

Table (1): Type, concentrations and symbols of the antibiotics used in the study (mg /c^3).

Antibiotics	Counter Symbol	Focus/disc
Cefixime	CFM	5
Amoxicillin	AMC	30
Ciprodar	CIP	10
Trim Methbrin	TMP	10
Azithromycin	AZM	15
Nalidixic acid	NA	30

Antibiotics Sensitivity test

The inhibitory activity against the bacteria used under study was tested by the method of diffusion by digging the sensitivity test at a rate of three replications for each germ, as holes were prepared in the nutrient medium with a diameter of (7 mm) and the bacterial suspension was prepared at the age of 16-14 hours and then the bacterial suspension was transferred to the nutrient medium in comparison with the MacFarland tube The standard amount equivalent to (108 cfu/ml×1.5), and by relying on a sterile cotton swab to spread the suspension on the surface in a consistent manner, then the dishes were placed in the incubator at a temperature of 37°C for 30 minutes to imbibe, then 0.1 microliter was placed in the hole from each Extract and at different concentrations (400, 200, 100) mg/cm, and the dishes were incubated at 37°C for 16 hours, after which a measurement of the diameter of the inhibition zone was taken [14,15].

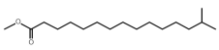
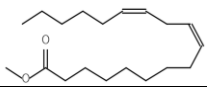
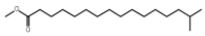
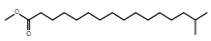
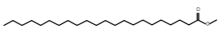
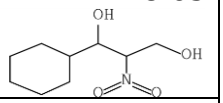
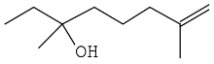
Results and Discussion

Identification of the active compounds

Diagnosis of some fatty acids using gas chromatography-mass spectrometry (GC-MS)

Using GC-MS technology to diagnose the fatty acids present in the petroleum ether extract of cumin seeds after the soaponification process The analysis showed the following standard compounds of acids: Octadecadienoic acid, Octadecenoic acid, Pentadecanoic acid, Hexadecanoic acid, Docosanoic acid as shown in Figure (1-9) and Table (2). The results of the diagnosis after the soaponification process showed that the presence of Octadecadienoic acid is the highest at a retention time of 17.306/min, followed by the rest of the acids, while Docosanoic acid has thelowest concentration of 0.09% with a retention time of 21,244/min compared with the rest of the fatty acids mentioned above.

Table 2. Identification of terpene compounds separated from cumin seeds by GC- MS.

Peak	R time	Area	Area%	Name	Type
1	15.365	162.77043	4.37	Pentadecanoic acid, 14-methyl-, methyl	Ester 
2	17.306	279426567	74.99	9‹12- Octadecadienoic acid (Z,Z)-, methyl	Ester 
4	17.519	4457428	1.20	Hexadecanoic acid, 15-methyl-, methyl	Ester 
5	19.432	596483	0.16	Hexadecanoic acid, 15-methyl-, methyl	Ester 
6	21.244	347705	0.09	Docosanoic acid, methyl	Ester 
7	22.931	916864	0.25	Cyclohexyl-2-nitropropane-1,3-diol	Sesquiterpe-noids 
8	23.986	3057025	0.82	Isocitronellol	Monoterpe-noids 

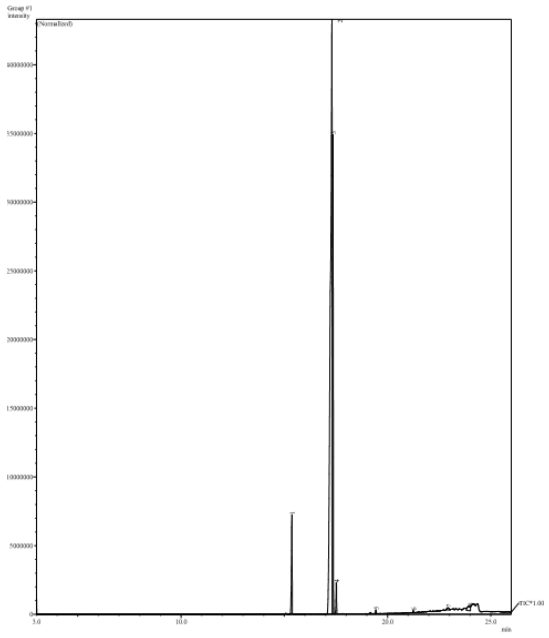


Figure (1): The schematic diagram of cumin fatty acid diagnosed by gas chromatography-mass spectrometry (GC-MS)

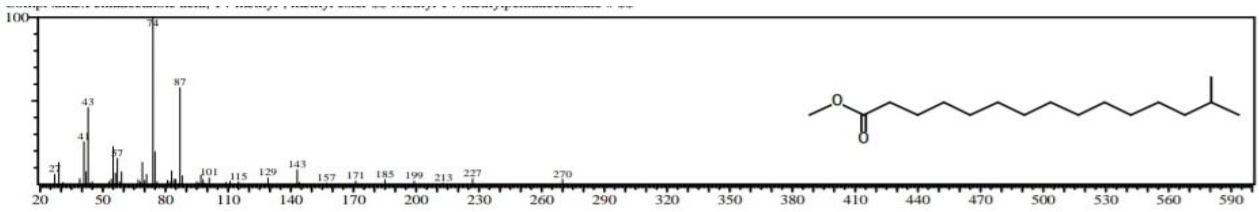


Figure (2): curve of Pentadecanoic acid with GC-M technology.

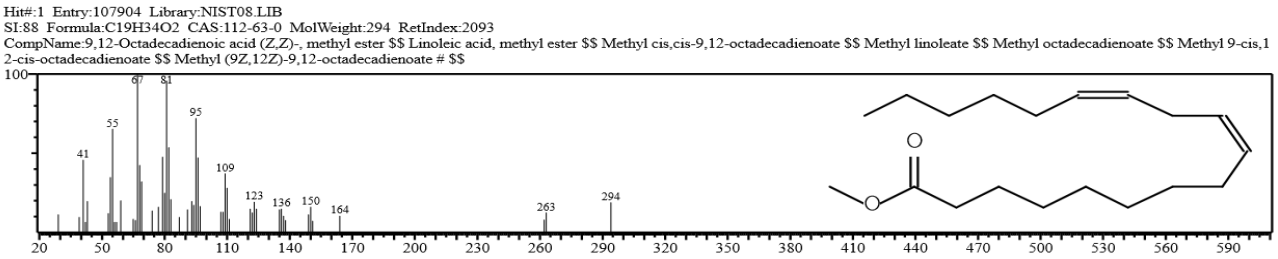


Figure (3): octadecadienoate Curve by GC-MS.

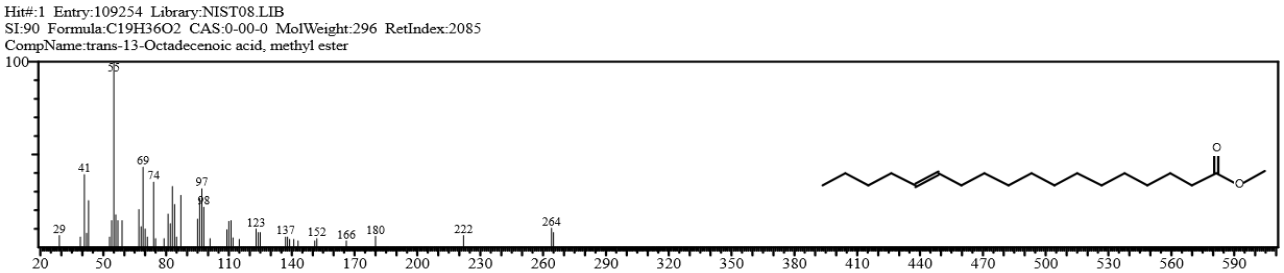


Figure (4): Curve - Octadecenoic acid by GC-MS.

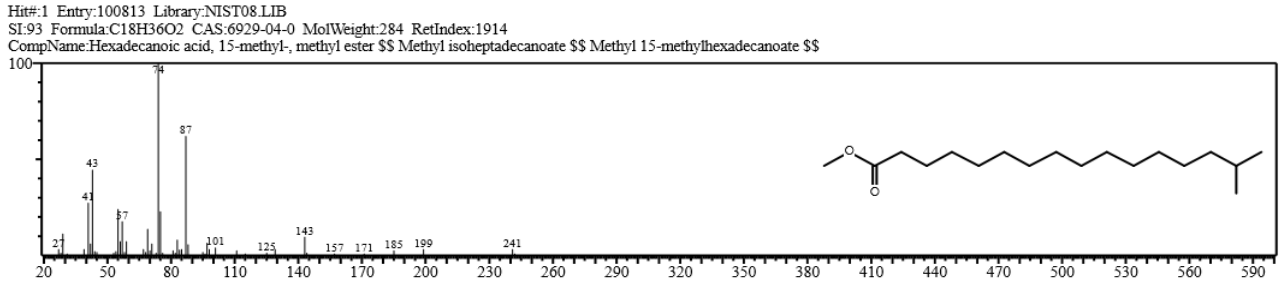


Figure (5): Hexadecanoic acid Curve by GC-M

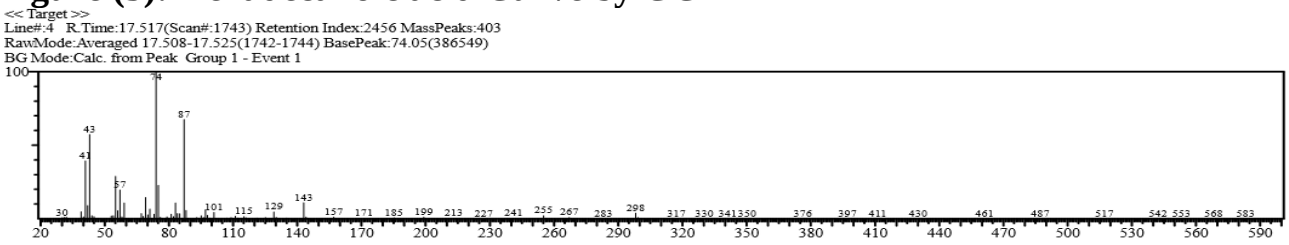


Figure (6): isoheptadecanoat Curve by GC-MS

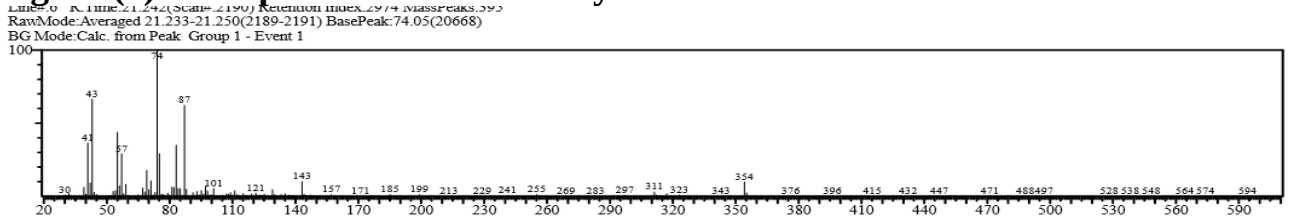


Figure (7): Docosanoic acid Curve by GC-MS

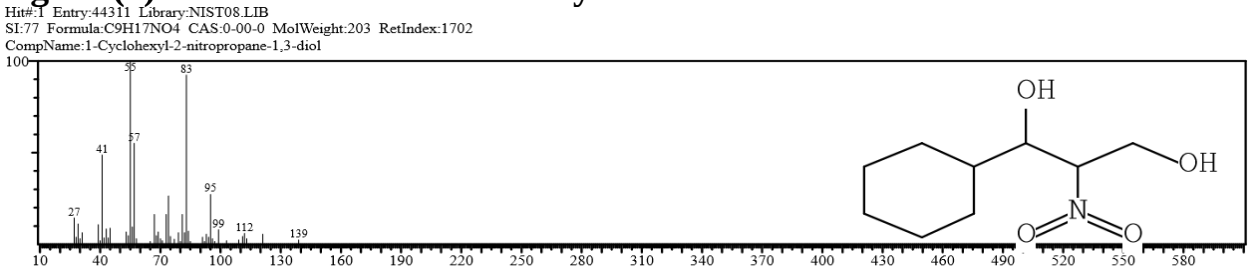


Figure (8): Cyclohexyl-2-nitropropane-1,3-dio l Curve by GC-MS

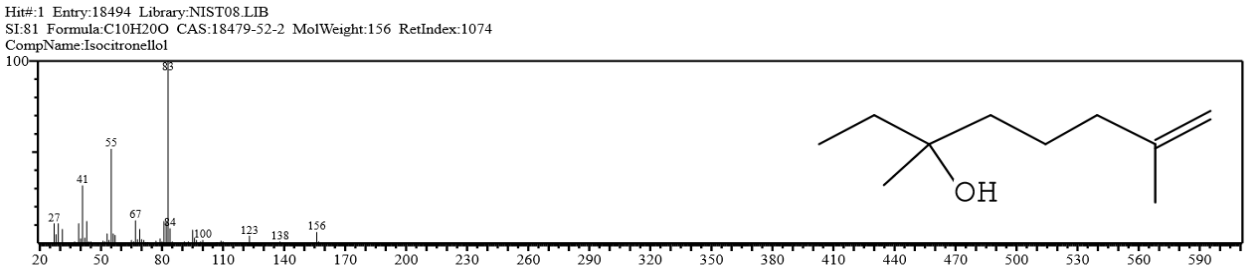


Figure (9): Isocitronellol Curve by GC-MS

In addition to the rest of the fatty acids resulting from the crude petroleum ether extract of cumin seeds after the soaping process, the fatty acid Octadecadienoic acid, known as oleic acid or Omega-6 unsaturated, works to reduce inflammation in the body and maintain heart health and blood pressure. As for Pentadecanoic acid, it is one of the Saturated fatty acids with a straight chain containing fifteen carbon atoms and is used in the soap industry for its ability to remove unwanted substances and

cosmetics While Docosanoic acid is a type of Omega-3 acid and has several benefits, the most important of which is reducing inflammation, reducing the risks of heart disease, diabetes and premature births, and working to support brain functions in infants. Hexadecanoic acid is a long-chain saturated fatty acid consisting of sixteen atoms. Carbon plays an important role in supporting the cell membrane as well as helping the body store energy to facilitate metabolism and act as an antioxidant that protects against free radical damage and signs of aging and helps to remove dust, sweat and oils from the skin by combining dirt and oil particles before and therefore it is used as an emollient when applied to the skin and is used in the manufacture of cosmetics, creams or bath oils and its ability to soften the skin and help it retain moisture by forming a greasy layer that prevents water, causing slows water loss through the skin [16].

The results of a similar study came with the researcher [17] The results of the diagnosis of the methanolic extract of cumin leaves, when analyzed using GC-MS technique, revealed the presence of nine pharmacologically active compounds, which are major compounds important for the treatment of various diseases, and of these compounds is hydroxylamine O-decyl is a compound The main one has an antioxidant effect, and heptafluoro acid has a strong insecticidal activity against *Sitophilus zamias and Tribolium castaneum*.

The results of the current study were similar to that of the researcher [18] due to a number of compounds produced from the methanolic extract of cumin seeds. These compounds are usually therapeutically active and have an inhibitory effect against pathogenic bacteria. 31 major compounds were detected using GC-MS technology.

Gas liquid chromatography (GlC) separation and characterization of cuminaldehyde from cumin seed volatile oils

Gas chromatography charts were obtained, through which the retention time of the compound Cuminaldehyde was determined compared to the standard retention time. A study [19] confirmed the volatile oil of cumin seeds using GlC technique, as the results showed the presence of cuminaldehyde, which is responsible for the distinctive smell of cumin, in addition to the inhibitory effect against some pathogenic bacteria As shown in the table (3) and figures (8,9).

Table (3): diagnosis of cuminaldehyde by the GLC device

Compound name	Standard R. time/min	R. time/minute
Cuminaldehyde	3.088	2.940

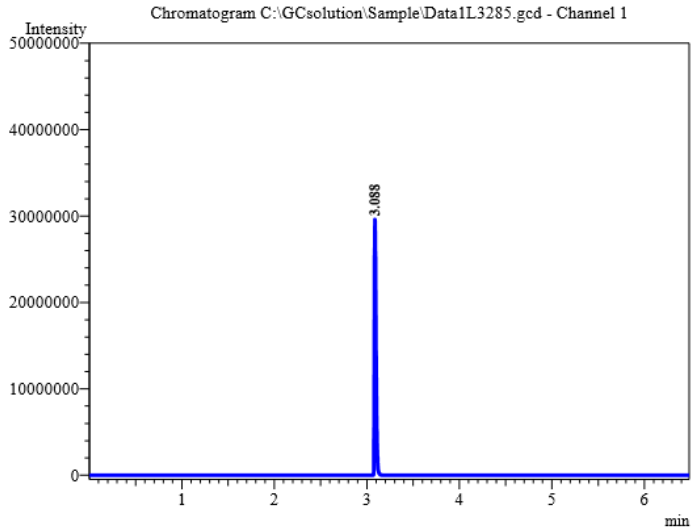


Figure (10): Tandards retention time of cuminaldehyde.

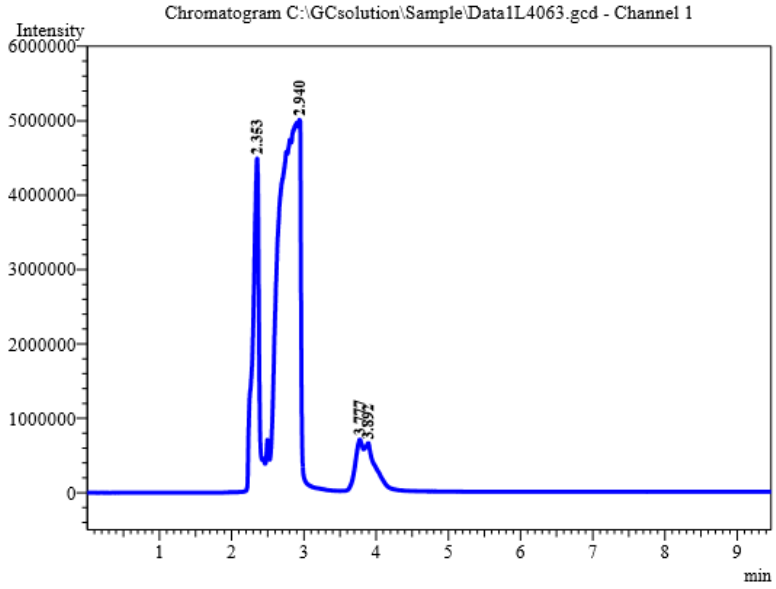


Figure (11): Retention time of cuminaldehyde

The focus of pathogenic antibacterial activity Inhibitory effect of fatty acids separated from cumin seeds against the bacteria used under study

The results showed that the fatty acids separated from cumin seeds gave the highest inhibition zones against *Staph.aureus* at a concentration of 400 mg/cm^3 with an effect of 15 mm for a bacterium, followed by a concentration of 200 mg/cm^3 by 14 mm and finally a concentration of 100 mg/cm^3 with an effect of 13 mm compared with the antibody Ciprodar, while E.coli had the highest concentration of 400 mg/cm^3 with an effect of 17 mm, followed by a concentration of 100 mg/cm^3, which had an effect of 14 mm, and finally, the effect of 13 mm came with a concentration of 200 mg/cm^3 compared to Ciprodar and Azithromycin antibiotics.

The highest concentration of *Agro.rhizogenes* came at a concentration of 400 mg/cm^3 by 19 mm, followed by the two concentrations 200 and 100 mg/cm^3 and

their effect was 12.17 mm respectively compared to other antibiotics. This is evidence of the presence of a variety of beneficial biological properties that play a role in maintaining On human health, there are many possible reasons why fatty acids are strong antioxidants and show different physiological activities so that fatty acids disrupt the structure of the lipid bilayer, which is a secondary membrane layer that fatty acid compounds work to destroy and then lead to cell death and kill the germ as well. It is shown in Table (4) and Pictures (1).

The results of the current study were similar to the study of [20]. The crude petroleum ether extract of cumin showed high levels of antibacterial activity, while the researcher [21] showed the inhibitory action of the crude petroleum ether extract of cumin seeds and showed an effective inhibitory effect against several Types of positive and negative bacteria and this is similar to the result of our current study[22],[23].

Table (4): Effect of cumin fatty acids against the bacteria used under study with different concentrations.

Concentration Mg/c^3	Types of Bacteria		
	Staph.	***E. coli***	***Agro.***
400	15 bcd ±1.5	26 a ±1.5	19 b ±1.1
200	14 cd ±2	13cd ±2	17 bc ±2
100	13 dc ±1	12 d ±2	12 d ±2.5

- Average diameter of the circle using three isolates for each bacterium.
- Different letters horizontally mean that there are significant differences at the
- level of significant difference ≤0.01 P.
- The diameter of the damping circle is measured in mm.
- (--) indicates lack of inhibitory activity.
- Data analyzed using Standered Error(±)

Inhibitory effect of volatile oils separated from cumin seeds against the bacteria used under study

The volatile oil separated from the seeds of the cumin plant showed a high inhibitory activity against the bacteria used under study, as it achieved the highest effect against *Staph.aureus* bacteria by 400 mg / cm^3 at the highest inhibition diameter of 37 mm, followed by other concentrations of 200 and 100 mg / cm^3 affected by 30.28 mm, respectively, while the oil proved its inhibitory effect on *E.coli* bacteria, and the highest inhibitory diameter was 42 mm by 400 mg/cm^3, followed by a concentration of 200 mg/cm^3 and at an inhibitory diameter of 40 mm, and the lowest concentration was given at 100 mg/cm^3 with an inhibition area of 31 mm. For *Agro.rhizogenes* bacteria, the concentration was 400 mg/cm^3, which gave the highest inhibitory diameter of 34 mm. It is followed by other concentrations distributed respectively 200,100% at the inhibition area 23-20 mm as shown in Table (5+6) and

Pictures cuminaldehyde (2) in comparison with antibiotics. The reason for the effectiveness of volatile oils separated from cumin seeds contain important components such as pinene, Cymene terpinene, cuminaldehyde, oleoresin, thymol showed an inhibitory effect. It is one of the low molecular weight compounds that contribute to a variety of physiological functions.

The results of the researcher's study [24] indicated an evaluation of the counter effects on some types of pathogenic bacteria, and the sensitivity method was used with tablets by measuring the diameter of the inhibition zone. The results of this study indicated that cumin essential oil showed an inhibitory effect against the bacteria used in the study for that. Use in the field of treatment [25, 26].

Table (5): Effect of volatile oils cuminaldehyde separated from cumin seeds against bacteria *S. aureus, E. coli, A. rhizogenes* in different concentrations

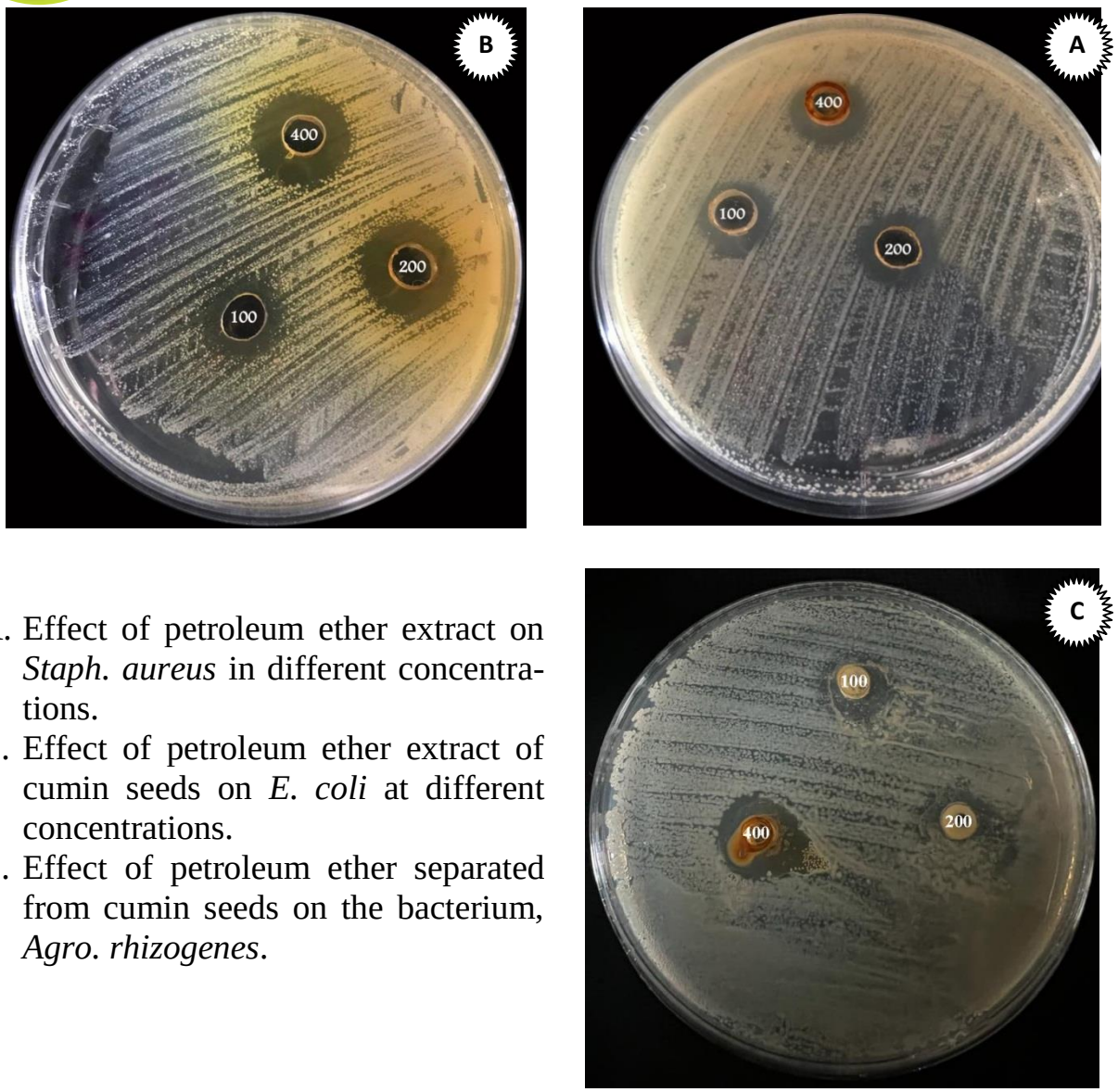
Concentration Mg/c^3	Types of Bacteria		
	S. aureus	***E. coli***	***A. rhizogenes***
400	37b ±1.5	42a ±1.7	34b ±1
200	30c ±1.5	40a ±2	23d ±1.5
100	28c ±1.5	31c ±2.0	20e ±0.5

- Average diameter of the circle using three isolates for each bacterium.
- Different letters horizontally mean that there are significant differences at the level of significant difference ≤0.01 P.
- The diameter of the damping circle is measured in mm.
- (--) indicates lack of inhibitory activity.
- The numbers after (±) indicate experimental error.

Table (6): Effect of antibiotics

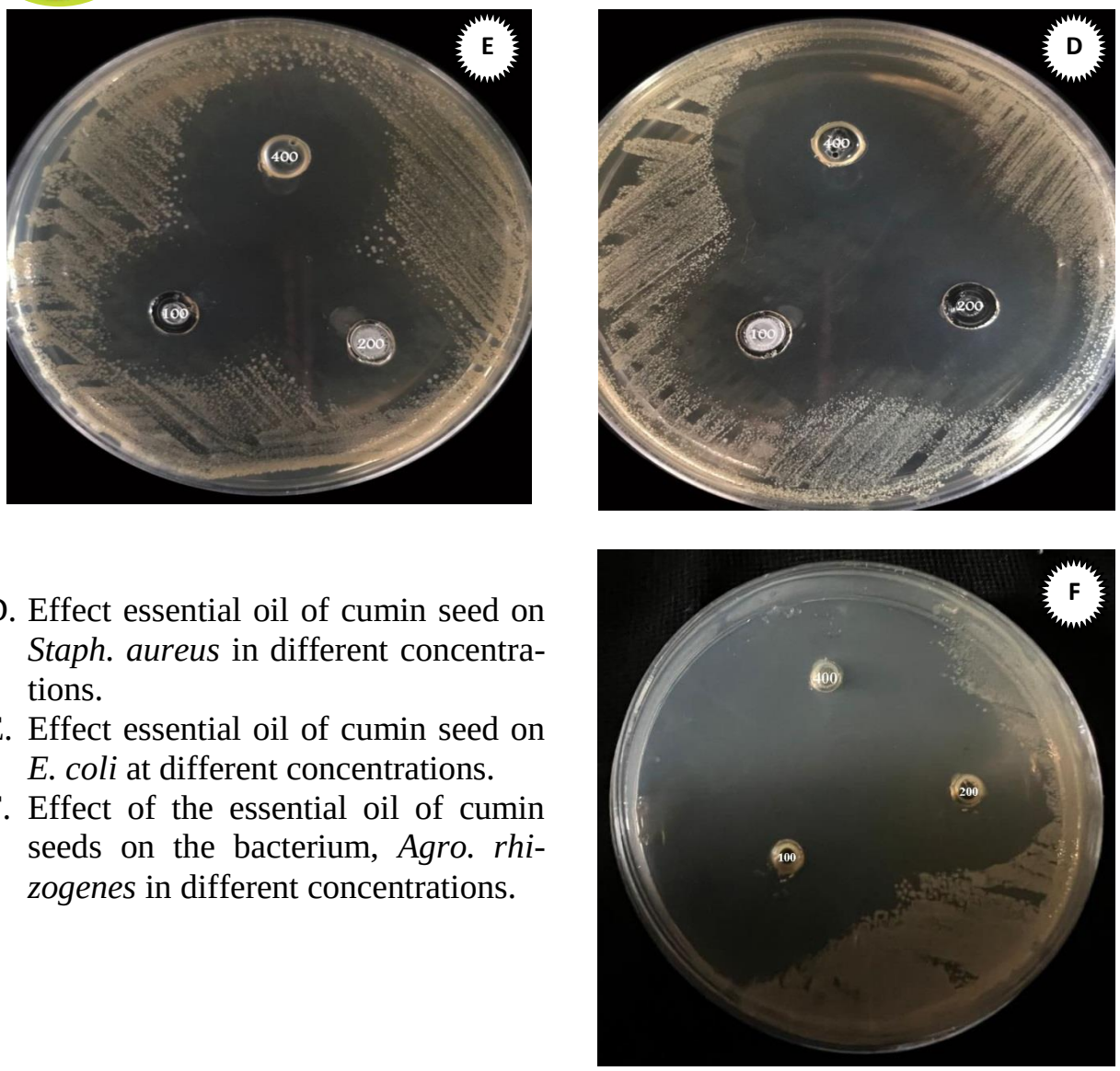
bacteria	NA	AZ M	TMP	CFM	AMC	CIP
S..aureus	14	14	0	0	0	12
E. coli	10	10	0	0	12	14
A. rhizogenes	11	11	12	0	13	10

- Bacterial number: 1st tube of McFarland standard solution.



A. Effect of petroleum ether extract on *Staph. aureus* in different concentrations.
B. Effect of petroleum ether extract of cumin seeds on *E. coli* at different concentrations.
C. Effect of petroleum ether separated from cumin seeds on the bacterium, *Agro. rhizogenes*.

Figure (12): inhibitory effect of the petroleum ether extract of cumin seed on the bacteria used under study of different concenterations (400,200,100 mg/c^3)



D. Effect essential oil of cumin seed on *Staph. aureus* in different concentrations.
E. Effect essential oil of cumin seed on *E. coli* at different concentrations.
F. Effect of the essential oil of cumin seeds on the bacterium, *Agro. rhizogenes* in different concentrations.

Figure (13): Inhibitory effect of essential oil cuminaldehyde of cumin seeds on the bacteria used under study at different concentrations (400, 200, 100 mg/cm^3)

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