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Phytochemical composition and antibacterial activities of some plant extracts

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ABSTRACT

This study was designed to examine the phytochemical composition of methanolic (M) and hexane extract (H) for *Coriandrum sativum* (CS) and *Cuminum cyminum* (CU) and analyzed by gas chromatography-mass spectrometry (GC-MS). Antibacterial activities were examined against four bacterial species (*Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*), highest antimicrobial activity was recorded against *Staph. aureus* and *P. aeruginosa* with inhibition zone 33 and 31.5 mm, respectively using the hexane extract for *C. sativum*, at a concentration of 100 µL. The best antimicrobial performance was obtained against *B. cereus* and *E. coli* with 26.6- and 28-mm inhibition zone, respectively using methanolic extract of *C. cyminum*, at a concentration of 100 µL. scanning electron microscopy (SEM) examination confirmed that both *Staph. aureus* and *B. cereus* cells densities in biofilms was inhibited and growth weakness of microbe's failure of the assembly system of bacterial cells and colony formation, enlargement of some cells, shrinkage of others, and deformation of the bacterial shape as a result of the effect of the two tested plant extracts. The study also found a change in zeta potential (ZP) value for *S. aureus* from 20.9 mV. to 0.193 mV. and *B. cereus* from -19.1 mV. to -0.282 mV., as a result of the effect of the extracts of *C. sativum* and *C. cyminum*, respectively. The GC-MS forty-two peaks of the compounds detected was shown in Chromatogram GC-MS analysis of hexane extract of *C. sativum* showed the presence of seventeen major peaks and the components corresponding to the peaks. The first set up peak was determined to be 1,3,5 Cycloheptatriene in area peak equal to 19.71% and methanolic extract of *C. cyminum* results showed that thirty-two peaks of component detected, the highest component was Benzaldehyde, 4-(1-methylethyl) in area peak equal to 25.73%.

1. INTRODUCTION

Coriandrum sativum popularly known as coriander, is used as a spice in food and medicinally, studies show that it has therapeutic effects, antioxidant, antimicrobial, and carminative, antispasmodic and relaxant. Coriander seeds contain an essential oil (EO) that possesses antibacterial properties against gram-negative and gram-positive bacteria, as well as various other species of microorganisms such as fungi, antibacterial activity for the EO obtained from coriander leaves against *Staphylococcus aureus*, *Bacillus spp*., *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Demonstrated antibacterial efficiency for the examined extract of *C. sativum* on *B. spp*. and *E. coli***. (Begnami *et al.,* 2010)***.*** This plant's leaves and seeds contain an (EO) that is safe and nontoxic. As a result, it is utilized as a fragrance, as well as a preservative and flavoring agent in the food sector and pharmaceutical productions. Linalool basically available in the EO of seeds **(Mandal and Mandal, 2015).** Currently, a lot of people favor using pharmaceuticals with natural ingredients **(Foudah *et al.,* 2021).** The greatest and most popular spice condiment in Asia is cumin. Cumin seeds are used as common culinary spices and aromatic plants. All varieties of cumin are used as stimulants, astringents, carminatives, and treatments for indigestion, diarrhea, and flatulence in traditional and veterinary medicine. **(Swagato *et al.,* 2015)** All of these qualities make cumin a good choice for use as a protective agent in food packaging, especially for fresh foods that cannot be spiked or created with additives **(Petretto *et al.,* 2018)**. Several reports have the antibacterial efficacy of *C. cyminum* extract against diverse species of microbes, pathogens and non-pathogens **(Shokri, 2014)**. P-isopropyl benzaldehyde, often known as cuminic aldehyde, is the primary component of cumin oil. Previous researches suggested that the primary ingredient, cuminic aldehyde, may be responsible for the antifungal and antibacterial effects **(Akrami *et al.,* 2015).** The compounds and active substances of spices have new and safe properties that can be developed as antimicrobials against antibiotic-resistant bacteria. Therefore, recent studies and research proposals are concerned used to study models membrane to bacteria, among them Zeta potential (ZP) measurements, ZP is another designation for electrokinetic potential **(Ferreyra *et al.,* 2019):;** It is an indirect way to assess the surface characteristics of bacteria since the ZP's effect and qualities depend on the net electric charges of the surface. This physical trait serves as the foundation for preserving ideal cell function. Additionally, it exerts a dominant influence on how bacteria adhere to surfaces and how they interact with the environment. Traditional surface property methods are challenging, however ZP values and rates are a simple procedure that reveals information regarding interfacial charges. This technique for determining the surface charge of particles is straightforward and repeatable, and it is being utilized more frequently across a wide range of academic subjects and practical areas to assess the nature of surface interactions between colloidal solution particles. **(Ferreyra *et al.,* 2021).** The aim of this study is to evaluate the antibacterial activity of hexane and methanol extracts of *Coriandrum sativum* and *Cuminum cyminum* and to find out the chemical composition of the most effective extract using GC-MS and SEM.

2.1. Materials:

Plant used: The study was done on two species of plants namely Coriandrum *sativum* and *Cuminum cyminum* **(Fig. 1.),** order Apiales and family Apiaceae. Dried seeds and fruit are the parts most traditionally used.

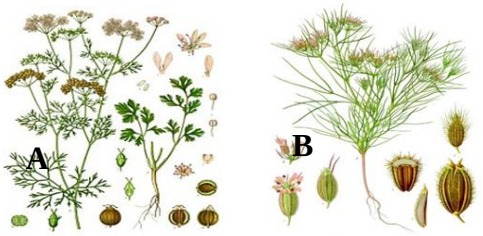

Fig 1.

Coriandrum sativum (A) and *Cuminum cyminum* (B)

Bacterial strains: Four bacterial strains were kindly supplied by Agric. Biotechnology Dept., Fac. of Agric., Damietta University, Damietta, Egypt. These bacterial strains were *Staph. aureus, B. cereus, E. coli* and *P. aeruginosa*. The bacterial strains were maintained on nutrient agar (NA) medium slant, at 5°C till use. Before use, the bacterial strains were sub-cultured on new slants of NA medium and incubated at 30°C for 18 h.

Chemicals and reagents: Para formaldehyde (2%) and glutaraldehyde (2.5%) fixative stock reagents: Value of 0.2 M 50 mL 0.2 M phosphate buffer at pH 7.4; para formaldehyde, 2.0 g; 25% aqueous glutaraldehyde, 10 mL of distilled water was completed to 100 mL **(Karnovsky, 1965), Osmium fixative stock reagent:** Osmium tetroxide 1% aqueous solution.

Ethanol solutions: Different series of grated ethanol consisting of 25%, 35%, 50%, 70%, 85% and 95% ethanol in distilled water and 100% stock reagent **(Kuo, 2007)**.

2.2 Methods:

Extraction of plant materials:

C. sativum (CS) and *C. cyminum* (CC) were collected from local markets in two governorates, Damietta and Dakahlia. Twenty grams of each sample were added to Erlenmeyer flasks along with 200 mL of each solvent (methanol CH_3OH or hexane C_6H_{14}). Mechanical shaking was used to extract the material at 27 °C for 72 hours. The residue was first filtered through filter paper (Whatman No. 1), then it was extracted two times. The combined extracts of each sample were then evaporated at room temperature (27° C), and they were dried in desiccators to a consistent weight. These two plant extracts' final dregs were used to investigate their antibacterial properties **(Roby *et al.*, 2013).**

Bacterial cultivation and antibacterial activities: Every bacterial strain was cultivated for one day at 30°C on NA slant. Each slant received five mL of sterile saline solution (0.09% NaCl). By gently pushing the microbes were made loose with a sterile inoculating loop. Bacterial cells were removed from the slant using a vortex mixer (original Vortex Genie 2 - Spain) for one minute. The antibacterial activity was assessed by using well diffusion techniques on Petri dishes containing 20 mL of NA medium. Using a sterile cotton swab, one bacterial strain was put onto each plate. Three tiny wells with a diameter of 6 mm were then drilled by a sterilized cork borer. Each well was filled with 25, 50, 75, or 100 µL of the four plant extracts that were being examined. All plates were incubated at 30°C. for 24 h. Then, inhibition zones appeared around the well were meticulously measured using a digital Vernier calliper **(Balouiri *et al.*, 2016).**

Scanning electron microscope:

Microbial cells specimens

A six mm-diameter well was drilled in agar containing the tested bacteria using a sterilized cork borer **(Mishra and Chauhan, 2016).**

Fixation: The samples were immersed in para formaldehyde (2%) and glutaraldehyde (2.5%) fixative at room temperature for 4h. The examined bacterial layer was kept uppermost for 40 min fixation at 27°C according to **Bancroft *et al*., (2013).**

Washing: After 4h fixation at room temperature, the specimen was rinsed three times for ten minutes each in 0.1 M phosphate buffer at pH 7.4 **(Mishra and Chauhan, 2016).**

Post-fixation: Samples were submerged for 2-4 hours at room temperature in 1% osmium tetroxide in 0.1 M phosphate buffer with a pH of 7.4, in a light-tight container. Osmium tetroxide solution, 1–2%: (1%) 0.1 M in 25 mL with 0.25 g OsO4 **(Kuo, 2007).**

Washing: After the last rinse, the samples were given three more phosphate buffer rinses, each lasting ten minutes **(Mishra and Chauhan, 2016).**

Dehydration: After being rinsed with distilled water, the samples were dehydrated for 10

minutes using a graduated ethanol series of 25%, 35%, 50%, 70%, 85%, and 100% **(Mishra and Chauhan, 2016).**

Critical Point Drying (CPD): As part of the sample preparation process for SEM, CPD is a technique for drying samples without collapsing or deforming the structure of wet, delicate specimens.

Mounting dried specimen on SEM stub: Following fixation and drying, specimens are mounted using double-sided tapes that are electrically conductive because they contain carbon **(Kuo, 2007).**

Metal sputter coating: The most popular technique is sputtering coating gold since it is quick and effective (**Bancroft *et al*., 2013).**

Viewing specimens in the scanning electron microscopy (SEM): Viewing specimens in SEM use JEOL JSM 6510LV scanning electron microscopy.

Zeta potential analysis: Malvern Zetasizer nanosizer was used for the dynamic light scattering approach to determine the zeta potential, Bacterial cultures that had been grown on the broth medium for 24 h. were used to make a colloidal solution of bacteria. Before performing the study, the acquired suspensions were centrifuged (MT-141 A, India, 8000 rpm, 10 min) and rinsed with sterile water (six times) to remove any remaining media and clean the bacterial cells. A volume of 0.5 mL of the stock bacterial suspension was utilized for the electrophoretic assays. For zeta potential measurements, 10 mL of the phosphate buffer solutions (pH 7) and 5 mL of the stock bacterial solution were combined. The mixture was then vortexed before being placed to the Malvern polystyrene U-shaped cell **(Ferreyra *et al.*, 2021).**

Gas chromatography-mass spectrometry (GC-MS) analysis:

A Thermo scientific 1310 gas chromatograph system, Italy and mass spectrophotometer MS tsq 9000 Italy was used, the traditional steps were performed as shown by **Olivia *et al.*, (2021).**

3.RESULTS AND DISCUSSION:

Table (1) displays the impact of the four spices extracts on bacterial growth**.** The effect of antibacterial effect of *C. sativum* (CS) and *C. cyminum* (CC) on four examined bacterial strains namely *Staph. aureus, B. cereus, E. coli* and *P. aeruginosa* were tested.

The effects of the methanolic and hexane extracts of (CS) on growth of *Staph. aureus:*

The findings of preliminary experiments employing the well diffusion method on antibacterial activity against *Staph. aureus* is displayed in **Tab.1.** illustrated **Fig. 2.** Results show that using hexane extract of CS exhibited the greatest antibacterial activity against *Staph. aureus.* Where the highest inhibition value was at a 100 µL concentration when the diameter of inhibition zone was 33 mm. The lowest value was at a 25 µL concentration, features a 19 mm diameter shown.

Hexane extracts of coriander were the most effective extract for preventing bacterial growth and metabolic function of biofilm for all bacteria studied, notably for *Staph. aureus* **(Molina *et al.*, 2020).**

Table 1. Results of the antibacterial activities of methanolic and hexane extracts

Tested bacteria	Samples code	Diameter of clear zone (mm) of antibacterial after 24h incubation period at concenters of (µL)			
		25 µL	50 µL	75 µL	100
Staph. aureus	**HCS**	**19.0**	**21.0**	**30.0**	**33.0**
	MCS	**17.5**	**20.0**	**22.3**	**24.4**
	HCC	**0.00**	**11.0**	**16.70**	**18.0**
	MCC	**0.00**	**13.7**	**17.5**	**23.0**
B. cereus	**HCS**	**0.00**	**0.00**	**0.00**	**12.0**
	MCS	**00.0**	**00.0**	**18.0**	**27.0**
	HCC	**0.00**	**12.5**	**19.0**	**20.4**
	MCC	**0.00**	**11.5**	**22.5**	**26.6**
E. coli	**HCS**	**0.00**	**0.00**	**0.00**	**15.0**
	MCS	**0.00**	**20.0**	**23.0**	**28.0**
	HCC	**9.00**	**14.0**	**15.0**	**21.8**
	MCC	**0.00**	**18.6**	**25.8**	**28.0**
P. aeruginosa	**HCS**	**0.00**	**17.0**	**18.4**	**31.5**
	MCS	**17.0**	**21.5**	**28.0**	**31.0**
	HCC	**0.00**	**12.6**	**17.5**	**24.0**
	MCC	**14.7**	**18.0**	**19.0**	**24.0**

(**HCS**): hexane extract of *Coriandrum sativum,* (**HCC**): hexane extract of *Cuminum cyminum,* (**HCS**): Methanolic extract of *Coriandrum sativum* and (**MCC**) Methanolic extract of *Cuminum cyminum*

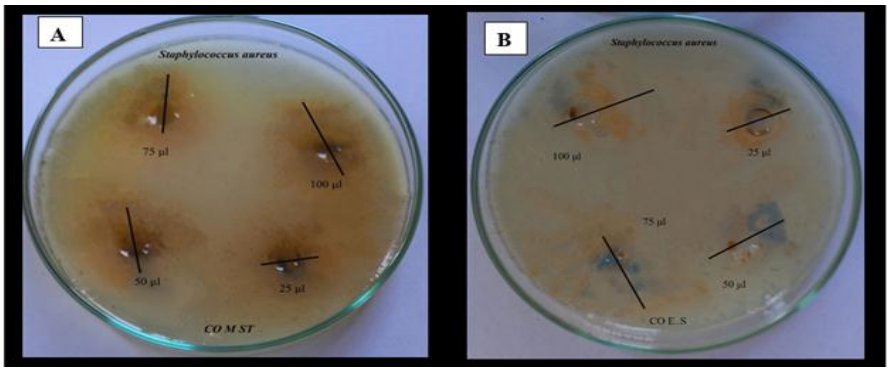

Fig. 2. The antibacterial activities of methanolic (A) and hexane (B) of CS extracts

SEM examination confirmed that *Staph. aureus* cell densities in biofilms inhibition and growth weakness and inhibition of cell division, failure of the assembly system of bacterial cells, enlargement of some cells, shrinkage of others, and deformation of the bacterial shape (Fig. 3).

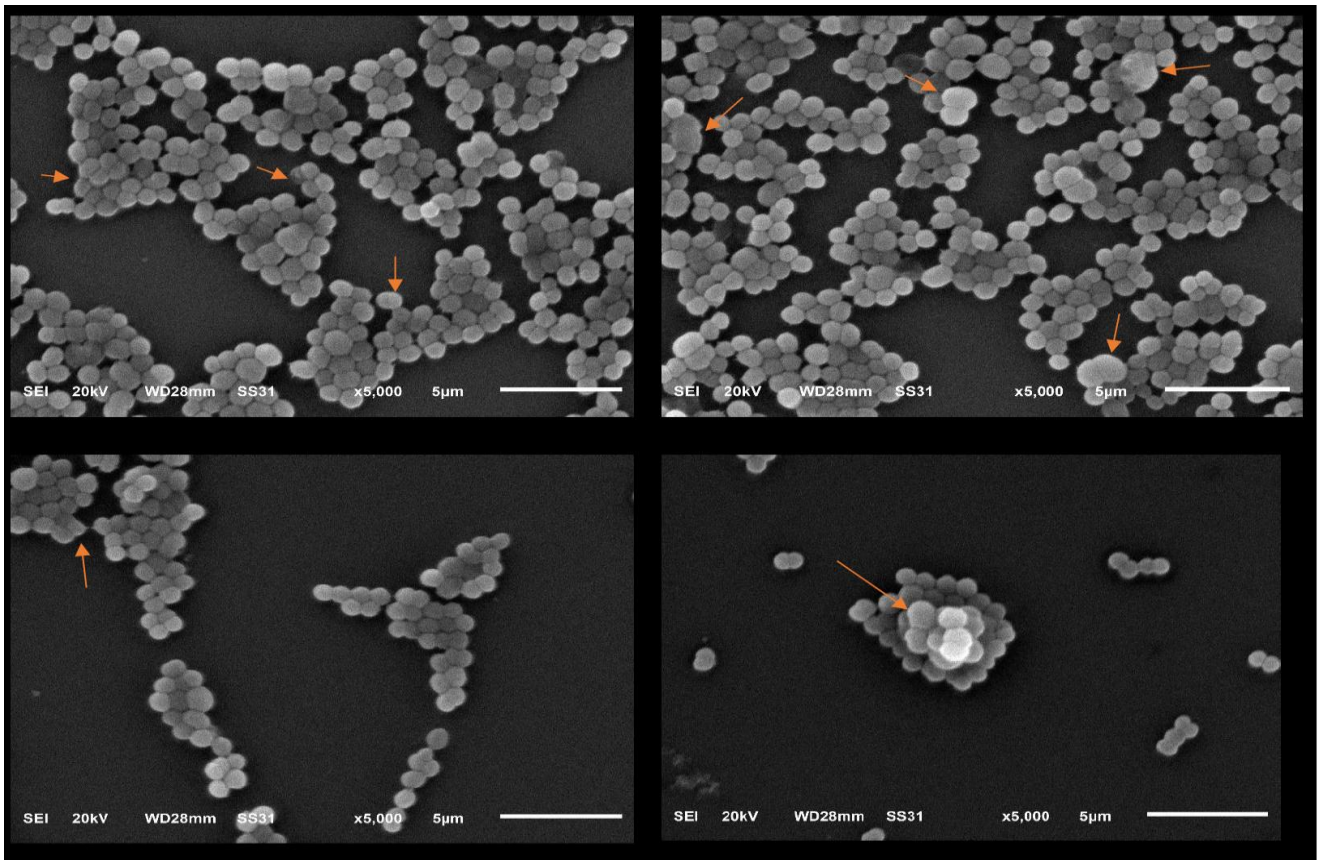

Fig. 3. Deformation of *Staph. aureus* shape as affected by hexane extract of *C. sativum.*

Effect of hexane extract of *C. sativum* on Zeta potential for *Staph. aureus:* Given the ZP measures technique's simplicity and ease of replication spanning many disciplines and sectors of application, it is increasingly employed to evaluate and contrast the nature of surface interactions between colloidal particles **(Bhattacharjee, 2016).** The results are shown in **Fig. 4**. showed the deterioration of ZP value because of the effect of the hexane extract of CS where it was before the treatment **-2٠.٩ mV** it was **0.193 mV**. After the treatment, teichoic acid-rich peptidoglycan layers are presented in *Staph. aureus* cell wall. The presence of anionic phosphate groups in the glycerol phosphate repeating units of teichoic acids is a cause of ZP precious's negative value **(Oh *et al.*, 2018).**

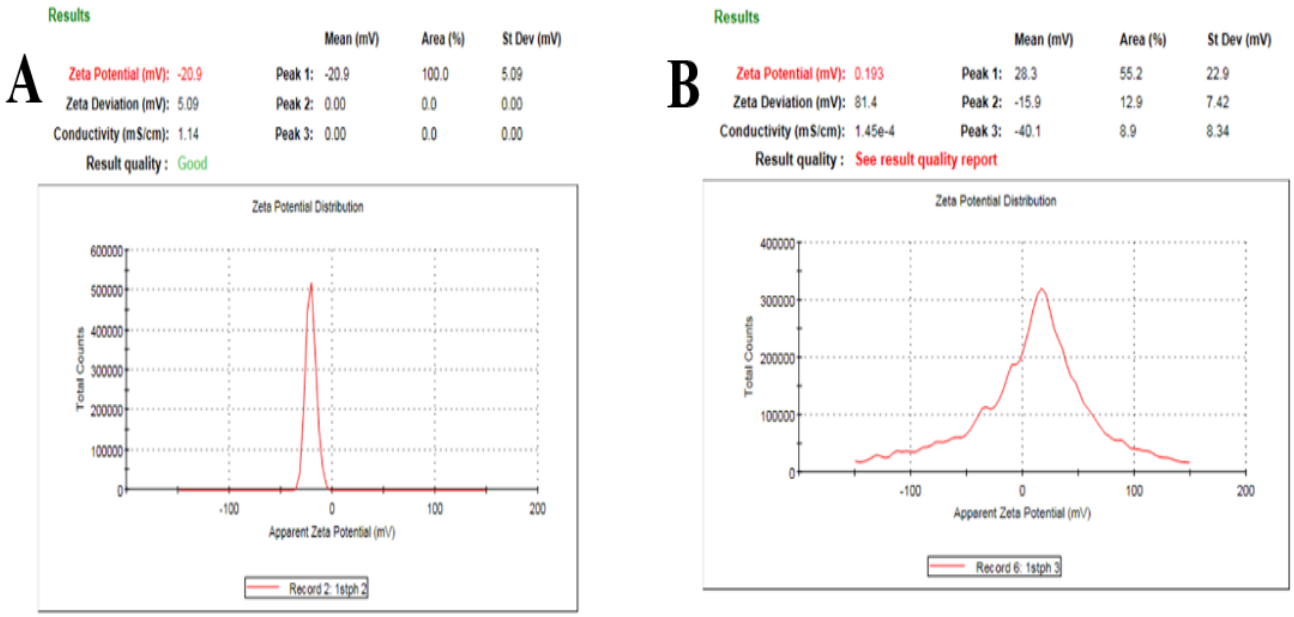

Fig. 4. Effect of hexane extract of *C. sativum* on Zeta potential for *Staph. aureus*

In a study on this subject, **Halder *et al.*, (2015)** showed that ZP in *E. coli* and *Staph. aureus* was altered by the surface-acting cationic agents for compounds. Additionally, they saw a rise in surface permeability, a proxy for membrane permeability, pointing to a potential connection between these two criteria. Both may eventually lead to diminished cell viability to remain. ZP considerably lowered when exposure time was increased to 30 minutes. The thick peptidoglycan coating in Gram positive bacteria is responsible for the resistance to the effect seen in *Staph. aureus*.

Effect of methanolic and hexane extracts of CS on growth of *B. cereus:*

The results obtained by examining the activity of CA extracts to inhibit the growth of *B. cereus* showed that the inhibitory effect was only in case of concentration of 100 µL in hexane extract when the diameter of the inhibition zone was 12 mm and concentration 75 µL when the diameter of the inhibition zone was 18 mm and 100 µL when the diameter of the inhibition zone was 27 mm in methanolic extract. Results in **Table 1.** and **Fig. 5.** Showed that the methanolic extract of. CA was better than the hexane extract by a percentage of 125% when it used at the highest concentrations (100 µL).

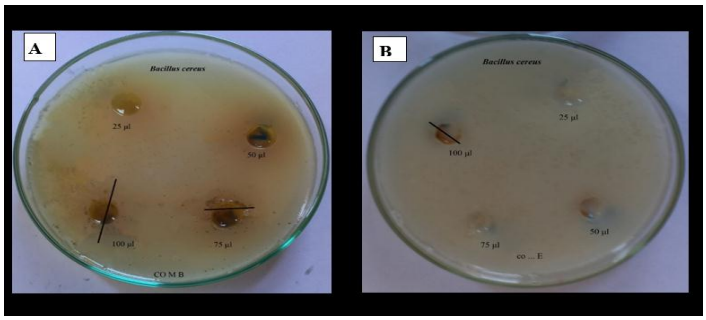

Fig. 5. The antibacterial activities of methanolic (A) and hexane(B) extracts of CS

Effect of methanolic and hexane extracts of CS on growth of *E. coli:*

Obtained results showed that the inhibitory effect was only in case of a concentration of 100 µL in hexane extract when the diameter of inhibition zone was 15 mm. In case of methanolic extract, the diameter of inhibition zone were 20 mm., 23 mm. and 28 mm. at a concentration of 50, 75, 100 µL for methanolic extract, respectively. As can be seen in **Table 1,** it was noted that the methanolic extract of CA was more effective than the hexane extract by a percentage of 186.67% when it used at the highest concentrations of 100 µL.

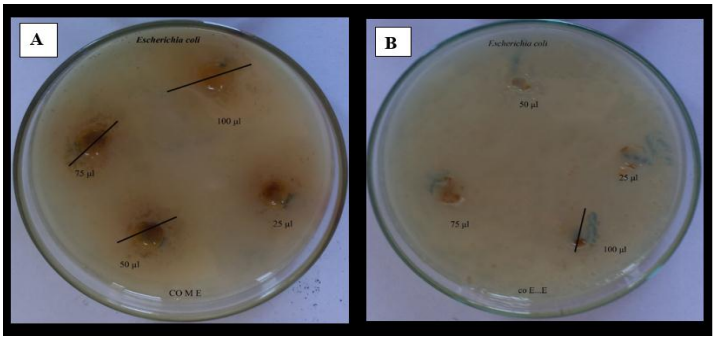

Fig. 6. The antibacterial activity of methanolic (A) and hexane(B) extracts of CS

Effect of methanolic and hexane extracts of CS on growth of *P. aeruginosa:*

Evaluation of antibacterial activity against ***P. aeruginosa*** is shown in Tab. 1. illustrated in **Fig. 7.** The best antibacterial performance was obtained against ***P. aeruginosa*** using the methanolic and hexane extracts for CS, where the highest inhibition value was at a concentration of 100 µL when the diameter of the inhibition zone was 31 and 31.5 mm, respectively. The lowest value was at a concentration of 25 µL, which showed a not detected diameter for hexane extract. It was noted that the methanolic extract of CS was most active than the hexane extract when it used at the lowest concentrations of 25 µL.

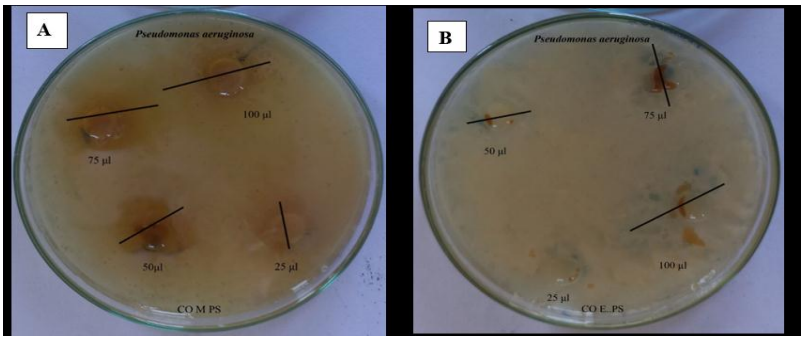

Fig. 7. The antibacterial activities of methanolic (A) and hexane(B) extracts of CS

GC-MS of hexane extract of CS:

Table 2 showing the GC-MS forty-two peaks of the compounds detected chromatogram of GC-MS analysis of hexane extract of CS showed the presence of seventeen major peaks and the components corresponding to the peaks were identified as follows, the first set up peak is to be 1,3,5-Cycloheptatriene (19.71%), the second peak indicated to be p-Xylene (14.87%). The next peaks considered to be Benzene, 1,3,5-trimethyl (3.05%), 1,6-Octadien-3-ol, 3,7-dimethyl (1.85%), oleic acid (2.16%). and octadecanoic acid, 2-[(1-oxohexadecyl) oxy]-1-[[(1-oxohexadecyl) oxy]

methyl] ethyl ester (3.99%). The compound 1,3,5-Cycloheptatriene (**Fig. 9)** was considered one of the main compounds found in high concentration (19.71%) and has proven its effectiveness as an antibacterial for a wide range of microbes, which is currently in usage **(Manikandan *et al.*, 2019).** It is noted for its presence in hexane extracts for *C. sativum and Capsicum annuum.*

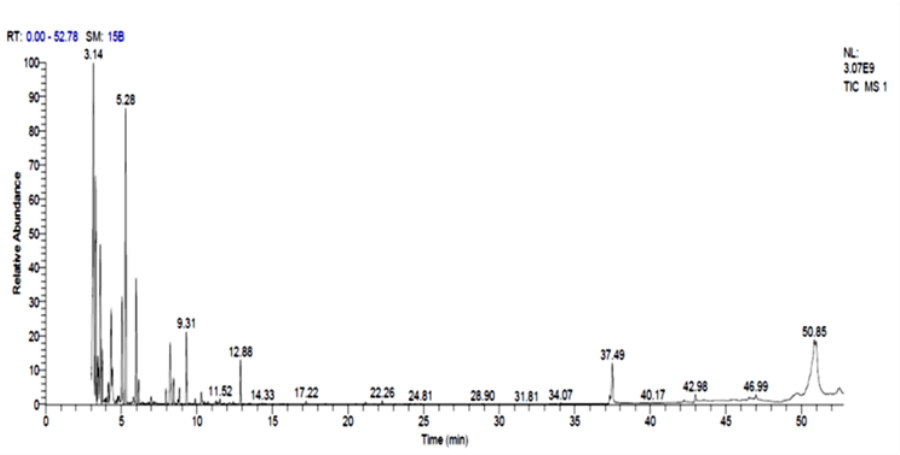

Fig. 8. Gas chromatography-mass spectrometry (GC-MS) of hexane extract of *C. sativum.*

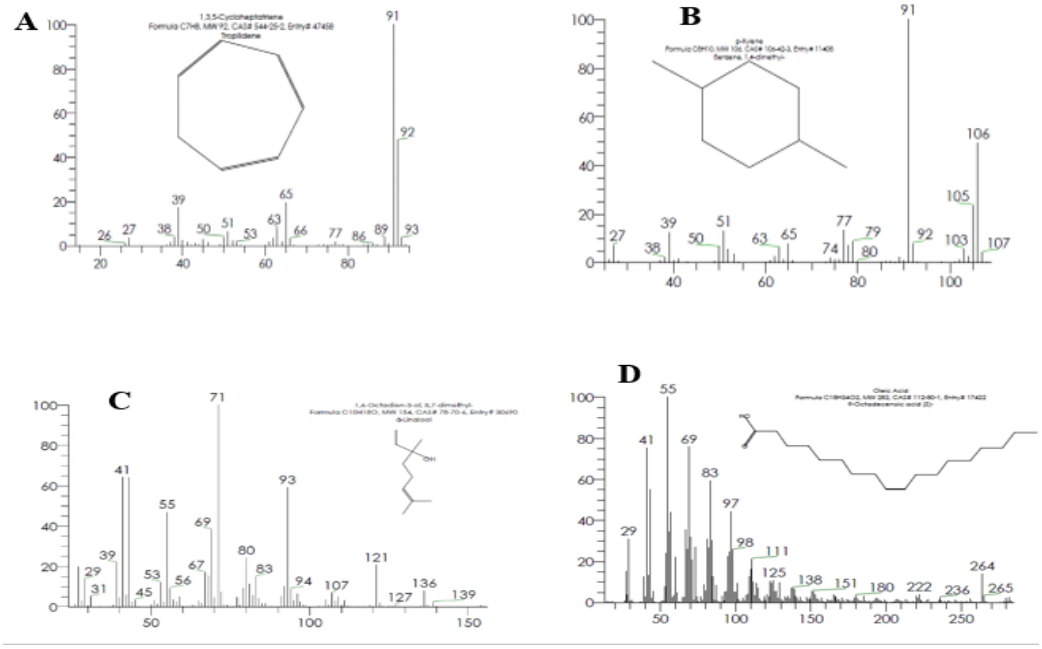

Fig. 9. Compounds structure and hit spectrum for 1,3,5-Cycloheptatriene (A), P-Xylene (B), 1,6-Octadien-3-ol, 3,7-dimethyl (C) and Oleic acid (d).

Table 2. Chemical compounds measured by Gas Chromatography-Mass Spectrometry of hexane extract of *C. sativum.*

N	RT	Compound name	Area %	Molecular Formula	Molecular Weight
1	3.15	1,3,5-Cycloheptatriene	19.71	C7H8	92
2	3.31	1,3-Dimethylcyclohexane, c &t	9.35	C8H16	112
3	3.43	Cycloheptane, methyl-	1.79	C8H16	112
4	3.49	Cyclopentane, 1-ethyl-2-methyl-, cis-	1.36	C8H16	112
5	3.6	Octane	8.24	C8H18	114
6	3.74	Cyclohexane, 1,3-dimethyl-, trans-	2.08	C8H16	112
7	3.86	Cyclopentane, butyl-	0.2	C9H18	126
8	3.92	1-Pentanol, 4-methyl-2-propyl-	0.19	C9H20O	144
9	4	Heptane, 2,4-dimethyl-	0.25	C9H20	128
10	4.14	Octane, 2,6-dimethyl-	0.81	C10H22	142
11	4.33	Cyclohexane, ethyl-	4.4	C8H16	112
12	4.4	1,1,4-Trimethylcyclohexane	1.34	C9H18	126
13	4.63	trans-1,2-Diethyl cyclopentane	0.2	C9H18	126
14	4.67	Cyclohexane, 1,3,5-trimethyl-, (1à,3à,5à)-	0.27	C9H18	126
15	4.84	Heptane, 2,3-dimethyl-	0.27	C9H20	128
16	5.04	Ethylbenzene	4.98	C8H10	106
17	5.28	p-Xylene	14.87	C8H10	106
18	5.8	1-Ethyl-3-methylcyclohexane (c, t)	0.28	C9H18	126
19	5.98	o-Xylene	5.2	C8H10	106
20	6.14	Heptane, 2,4,6-trimethyl-	1.01	C10H22	142
21	6.97	Benzene, 1-ethyl-2-methyl-	0.32	C9H12	120
22	7.95	N-Benzyl-2-phenethylamine	0.68	C15H17N	211
23	8.22	Benzene, 1-ethyl-3-methyl-	3.37	C9H12	120
24	8.45	Benzene, 1-ethyl-4-methyl-	1.13	C9H12	120
25	8.75	α-Pinene	0.19	C10H16	136
26	8.84	Benzene, 1,2,4-trimethyl-	0.67	C9H12	120
27	9.31	Benzene, 1,3,5-trimethyl-	3.05	C9H12	120
28	9.89	3-Carene	0.23	C10H16	136
29	10.28	Benzene, 1,2,3-trimethyl	0.51	C9H12	120
30	10.36	Benzene, 2-ethyl-1,3-dimethyl	0.13	C10H14	134
31	11.26	Benzene, 1-methyl-3-propyl-	0.19	C10H14	134
32	11.52	1,3,8-p-Menthatriene	0.26	C10H14	134
33	12.88	1,6-Octadien-3-ol, 3,7-dimethyl-	1.85	C10H18O	154
34	17.22	Benzaldehyde, 4-(1-methylethyl)-	0.14	C10H12O	148
35	22.26	Caryophyllene	0.16	C15H24	204
36	37.31	Oxacycloheptadec-8-en-2-one	0.29	C16H28O2	252
37	37.5	Oleic Acid	2.16	C18H34O2	282
38	42.98	2,3-Dihydroxypropyl elaidate	0.38	C21H40O4	356
39	46.98	9-Octadecenoic acid (Z)-, tetradecyl ester	0.24	C32H62O2	478
40	50.85	Octadecanoic acid, 2-[(1-oxohexadecyl) oxy]-1-[[(1-oxohexadecyl) oxy] methyl] ethyl ester	3.99	C55H106O6	862
41	50.98	Octadecanoic acid 2-[(1-oxohexadecyl) oxy]-1,3-propanediyl ester	2.75	C55H106O6	862
42	52.52	Ethyl iso-allocholate	0.52	C26H44O5	436

The compound 1,3-Dimethylcyclohexane (9.35%) showed activity similar to that of ampicillin against *S. aureus* **(Ceramella *et al.*, 2022).** P-Xylene **Fig. 9** (14.87%) has antibacterial, antifungal and antioxidant properties **(Morah *et al.*, 2019)**. Linalool (1,6-Octadien-3-ol, 3,7-dimethyl). There are numerous findings in the literature on the antibacterial activities of linalool, which is a component of many essential oils and is shown in Fig. 8. Monoterpene is efficient against pathogenic bacteria, such as multi-drug resistant strains of *E. coli* O157:H7 or *P. aeruginosa*, whose treatment is frequently challenging due to their high level of resistance to therapeutic antibiotics **(Maczka *et al.*, 2022).** Oleic acid (**Fig. 9)** is a fatty acid that occurs normally in various animal and plant fats and oils that have antibacterial effect, particularly in inhibiting growth of several Gram-positive bacterial species. Oleic acid is classified as an antibiotic; oleic acid shows antibacterial activity against *Staph. aureus* **(Jumina *et al.*, 2019)**. The most effective extracts for preventing the development and metabolic activity of microbial biofilm, particularly for *Staph. aureus*, were coriander extracts in hexane. There are 11 chemical components found in coriander hexane, with oleic acid among the primary ones in the extract **(Molina *et al.*, 2020).**

Effect of methanolic and hexane extracts of CC on growth of *Staph. aureus*:

Results of investigating the ability of hexane and methanolic extracts of CC to prevent growth of *S. aureus* did not show results only at concentration 25 µL, while the rest of the concentrations showed antibacterial activity as the diameters were 11 mm, 16.7 mm and 18 mm for concentrations of 50 µL, 75 µL and 100 µL of the hexane extract, respectively, and 13.7 mm, 17.5 mm, and 23 mm, respectively, for the methanolic extract. Evaluation of antibacterial activity against *Staph. aureus* using well diffusion method are shown in Tab. 1. illustrated in **Fig. 10.** As can be seen in **Table 1,** it was noted that the methanolic extract of CC was better than the hexane extract by a percentage of ١٢٧.٨% when using at the highest concentrations of 100 µL.

Effect of methanolic and hexane extracts of CC on growth of *B. cereus*:

The results of testing the antibacterial activity of the hexane and methanolic extracts to inhibit the growth of ***B. cereus*** of CC did not show results only at concentration of 25 µL, while the rest of the concentrations showed antibacterial activity as the diameters were 12 mm, 19 mm and 20.4 mm for concentrations of 50 µL, 75 µL and 100 µL of the hexane extract, respectively. and 11.5 mm, 22.5 mm, and 26.6 mm, respectively, for the methanolic extract**.** The results of preliminary evaluation on antibacterial activities against ***B. cereus*** using well diffusion method are shown in **Fig. 11** As demonstrated in **Table 1** It was noted that the methanolic extract of CC was better than the hexane extract by a percentage ١30.4% whine it used at the highest concentrations (100 µL).

Effect of methanolic and hexane extracts of CC on growth of *B. cereus*:

The results of testing the antibacterial activity of the hexane and methanolic extracts to inhibit the growth of ***B. cereus*** of CC did not show results at concentration of 25 µL, while the rest of the concentrations showed antibacterial activities as the diameters were 12 mm, 19 mm and 20.4 mm for concentrations of 50 µL, 75 µL and 100 µL of the hexane extract, respectively, and 11.5 mm, 22.5 mm, and 26.6 mm, respectively, for the methanolic extract**.** The results of preliminary evaluation on antibacterial activities against ***B. cereus*** method are shown in **Fig. 11** As shown in **Table 1** it was noted that the methanolic extract of CC was better than the hexane extract by a percentage of ١30.4% whien it used at the highest concentrations (100 µL). SEM examination confirmed that *B. cereus* cells densities in biofilms were inhibited beside weakness of microbial growth and inhibition of cell division, failure of the assembly system of

bacterial cells and colony formation. Enlargement of some cells, shrinkage of others, and deformation of the shape of bacteria were also noticed **(Fig. 12).**

Zeta potential for *B. cereus* as a result of C. cyminum methanolic extract effect*:* ZP for the majority of bacteria revealed a negative value, which is most likely due to the dominance of negatively charged influential groups linked to peptidoglycan **(Ferreyra *et al.*, 2021).** The results shown in **Fig. 13** proved the deterioration of Zeta potential for *B. cereus* value because of the effect of the methanolic *C. cyminum* extract where it was **-19.1 mV** before the treatment **while** it was **-0.282 mV** after the treatment.

One of the key determination of the effect on bacteria is the molecular characteristics of the bacterial cell surface, the first and most important factor in the cohesion process of the surface charge **(Spriano *et al.*, 2017).** Measuring ZP is an accurate and important technique for studying the interactions between compounds. It is the most important preliminary steps that show the mechanism of action of most compounds that act as antibacterial agent other steps followed, properties of bacteria can be modified by adsorb some certain solutes resulting in surface-level heterogeneous electrical and chemical conditions. ZP was used to study and understand the action's technique and interaction of antibacterial compounds and to collect more information about these functions in addition to the biophysical properties. **Freire *et al.*, (2011)** showed the mathematical description for the relationship of ZP to bacteria and compounds that are used as antibacterial and their selectivity and the determination of physical and chemical factors for different membrane models.

Effect of methanolic and hexane extracts of CC on the growth of *E. coli*:

Findings from experiments examining the ability of hexane and methanolic extracts to prevent the growth of *E. coli* of CC did not show results only at concentration of 25 µL for methanolic extract, while the rest of the concentrations showed antibacterial activity as the diameters of inhibits zone were 9 mm, 14 mm., 15 mm and 21.8 mm for concentrations of 25 µL, 50 µL, 75 µL and 100 µL of the hexane extract, respectively. Also, 18.6 mm, 25.8 mm, and 28 mm, respectively, for the methanolic extract for concentrations of 50 µL, 75 µL and 100 µL. The results of antibacterial activities against *E. coli* are shown in Tab. 1 and illustrated in **Fig.14** As can be seen in **Table 1**; It was noted that the methanolic extract of CC was most active than the hexane extract by a percentage 128.44% when it used at the highest concentrations of 100 µL.

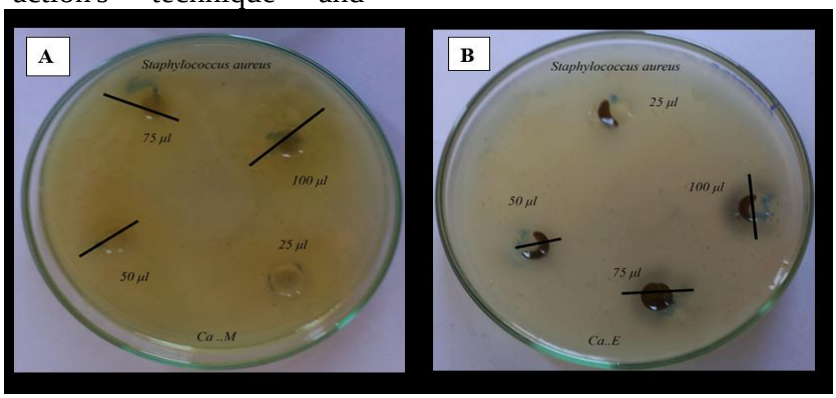

Fig. 10. The antibacterial activities of methanolic (A) and hexane (B) extracts of CC

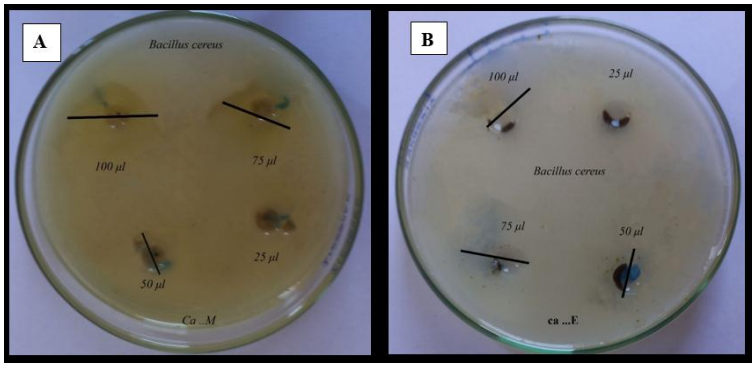

Fig. 11. The antibacterial activity of methanolic (A) and hexane(B) CC extracts

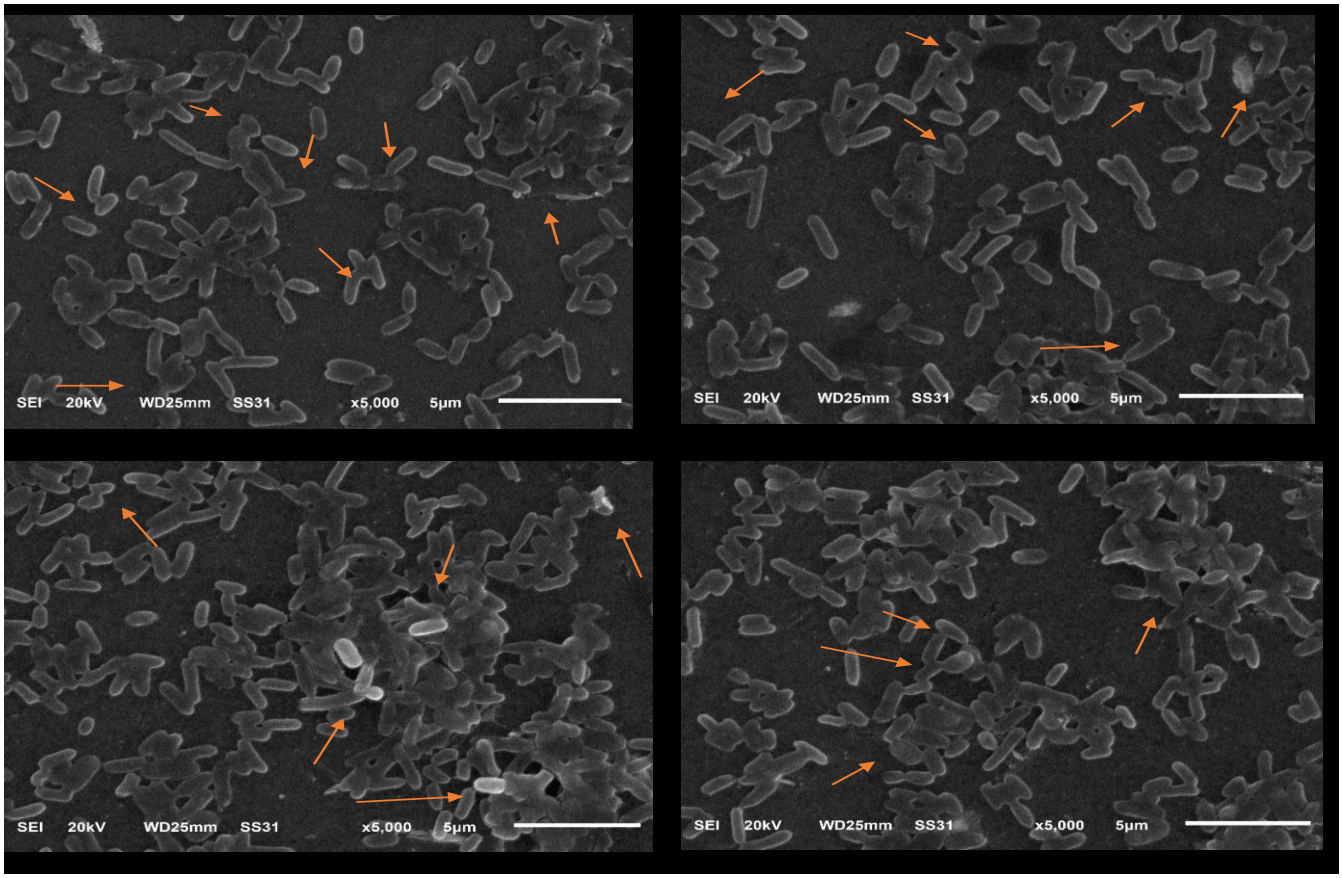

Fig. 12. Deformation of *B. cereus* shape as affected by methanolic extract of CC.

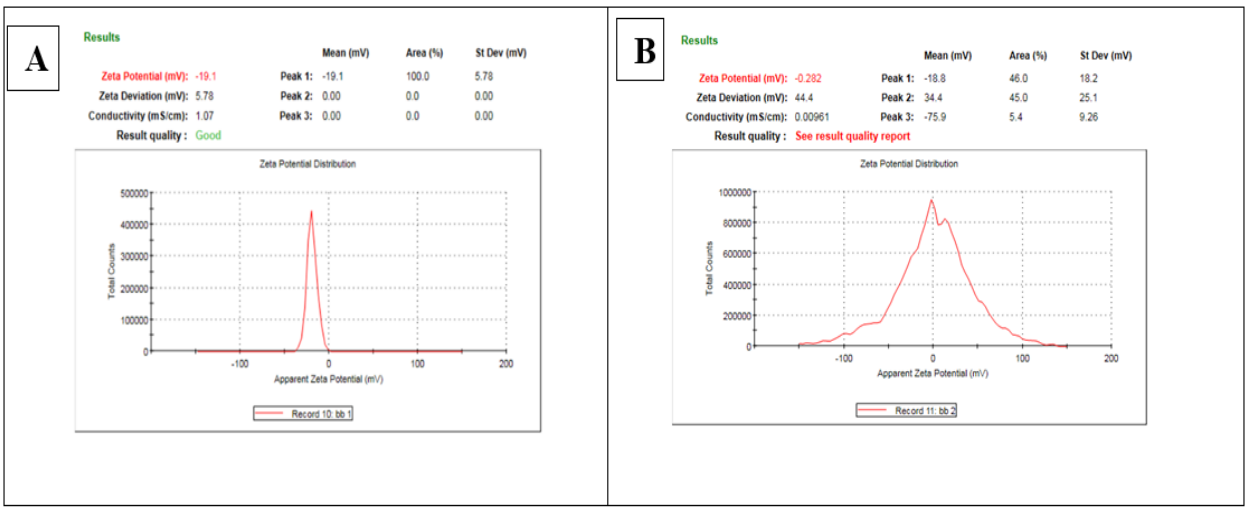

Fig. 13. Effect of the methanolic extract of CC on Zeta potential for *B. cereus.*

Effect of methanolic and hexane extracts of CC on the growth of *P. aeruginosa:*

Evaluation of antibacterial activity against ***P. aeruginosa*** is shown in Tab. 1. illustrated in **Fig. 15.** As is evident in **Table 1,** the best antibacterial performance obtained against ***P. aeruginosa*** using the hexane extract for CC, Where the highest inhibition value was at a 100µL concentration when the diameter of inhibition zone was ٢٤ mm. The lowest value was at a 25 µL concentration, which showed a not detected inhibition of hexane extract. It was noted that the CC methanolic extract was better than the hexane extract when it used at the lowest concentrations (25 µL). It was noted that the methanolic extract of. ***P. nigrum*** had similar effects and results for the hexane extract

GC-MS of hexane extract of CC extract:

Chromatogram of CC methanolic extract results showed that thirty-two peaks of component detected in **Table 3. Fig 16** showed that the highest components are Benzaldehyde, 4-(1-methylethyl) (25.73%), 1-(p-tert-Butylphenyl) ethanol (7.23%), á-(2-Methoxy-4,6-dimethylphenyl) butyric acid, methyl ester (2.83%), Benzenepropanal,3-(1,1-dimethylethyl)-à-methyl (4.6%), Formic acid and 9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-,3-acetate (12.62%). Benzaldehyde, 4-(1-methylethyl) (Cuminaldehyde) (25.73%) (**Fig**. **17)**. Evaluation of cumin aldehyde activity has demonstrated antibacterial and biofilm efficacy against *Staph. aureus* and *E. coli* **(Monteiro-Neto *et al.*, 2020).** Ethyl iso-allocholate (5.02%) and oleic acid Studies have shown that it contains antibacterial capabilities **(Shah *et al.*, 2021).**

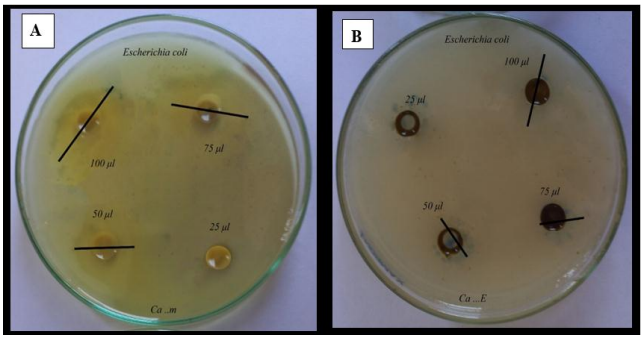

Fig. 14. The antibacterial activity of methanolic (A) and hexane(B) extracts of CC

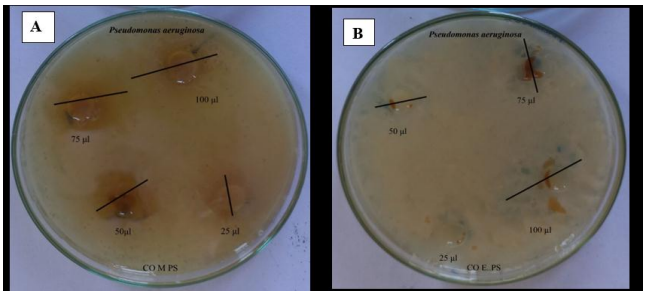

Fig. 15. The antibacterial activity of methanolic (A) and hexane(B) extracts of CC

Table 3 Chemical compounds obtained by Gas Chromatography-Mass analysis (GC-MS) of methanolic extract of CC.

N	RT	Compound name	Area %	Molecular Formula	M.W
1	3.09	Heptaethylene glycol	0.89	C14H30O8	326
2	10.26	Benzene, 1-methyl-2-(1-methylethyl)-	0.31	C10H14	134
3	10.33	Benzene, 4-ethyl-1,2-dimethyl-	0.53	C10H14	134
4	12.83	Tetradecane	0.7	C14H30	198
5	15.85	1,3-Cyclohexadiene-1-methanol,4-(1-methylethyl)-	0.4	C10H16O	152
6	17.05	Propanal, 2-methyl-3-phenyl	1.24	C10H12O	148
7	17.18	Benzaldehyde, 4-(1-methylethyl)-	25.73	C10H12O	148
8	18.47	2-Caren-10-al	0.42	C10H14O	150
9	18.69	Phenol, 2-methyl-5-(1-methylethyl)-	1.05	C10H14O	150
10	19.75	10,12-Octadecadiynoic acid	0.68	C18H28O2	276
11	20.52	Cyclohexene,4-isopropenyl-1-methoxymethoxymethyl	1.6	C12H20O2	196
12	20.73	1-(p-tert-Butylphenyl)ethanol	7.23	C12H18O	178
13	26.59	Globulol	0.94	C15H26O	222
14	26.78	á-(2-Methoxy-4,6-dimethylphenyl)butyric acid, methyl ester	2.83	C14H20O3	236
15	28.02	Benzenepropanal,3-(1,1-dimethylethyl)-à-methyl-	4.6	C14H20O	204
16	31.26	Pregan-20-one,2-hydroxy-5,6-epoxy-15-methyl-	0.52	C22H34O3	346
17	36.59	Linoleic acid ethyl ester	0.43	C21H36O4	352
18	36.72	6,9,12,15-Docosatetraenoic acid, methyl ester	0.47	C23H38O2	346
19	37.24	Docosahexaenoic acid, 1,2,3-propanetriyl ester	0.6	C69H98O6	1022
20	39.79	1-Monolinoleoylglycerol trimethylsilyl ether	1.07	C27H54O4Si2	498
21	40.17	Formic acid,7-hydroxymethyl-4-isopropyl-1-methyl-bicyclo[3.2.1]oct-6-en-8-ylmethyl ester	12.62	C15H24O3	252
22	42.18	Glycine, N-[(3à,5á)-24-oxo-3-[(trimethylsilyl)oxy]chola n-24-yl]-, methyl ester	1.15	C30H53NO4S i	519
23	44.54	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethyl silyl)oxy]cholan-24-yl]-, methyl ester	0.97	C36H69NO6S i3	695
24	47.56	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca methyl	1.01	C16H50O7Si8	578
25	48.4	Retinol	0.68	C20H30O	286
26	49.29	7,8-Epoxylanostan-11-ol, 3-acetoxy	1.2	C32H54O4	502
27	49.65	Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl	2.15	C27H42O4	430
28	50.07	Rhodopin	1.89	C40H58O	554
29	50.81	Ethyl iso-allocholate	5.02	C26H44O5	436
30	50.93	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-,3-acetate	12.85	C31H52O3	472
31	51.46	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-trio l, (3á,5Z,7E)-	6.17	C27H44O3	416
32	52.03	1-Monolinoleoylglycerol trimethylsilyl ether	2.04	C27H54O4Si2	498

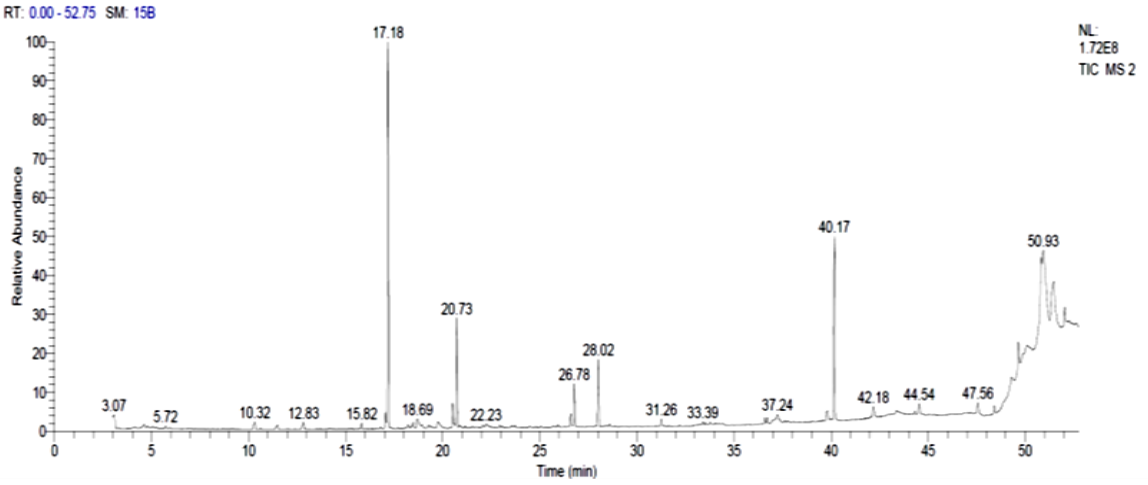

Fig. 16 Gas chromatography-mass spectrometry (GC-MS) of methanolic extract of CC.

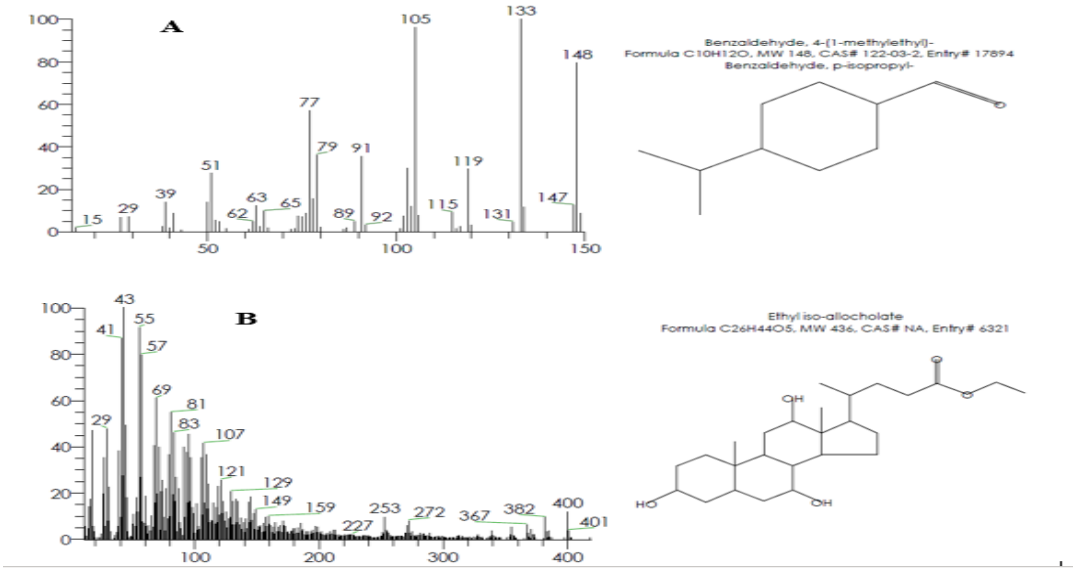

Fig. 17 Compound structure and hit spectrum for benzaldehyde, 4-(1-methylethyl) (Cuminaldehyde) (A) and ethyl iso-allocholate (B)

4. CONCLUSION:

The results showed the antibacterial activities and chemical compounds of the extracts of Coriandrum sativum and Cuminum cyminum. The study also found a change in zeta potential of examined bacteria, as well as the results were confirmed by using scanning electron microscope.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest**.**

AUTHORS CONTRIBUTION:

All authors developed the concept of the manuscript. El-Gayar wrote the manuscript and achieved the experimental work and measurements. All authors checked and confirmed the final revised manuscript.

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الملخص العربى

التحليل الكيميائي وقوة النشاط الضد بكتيري لبعض المستخلصات النباتية

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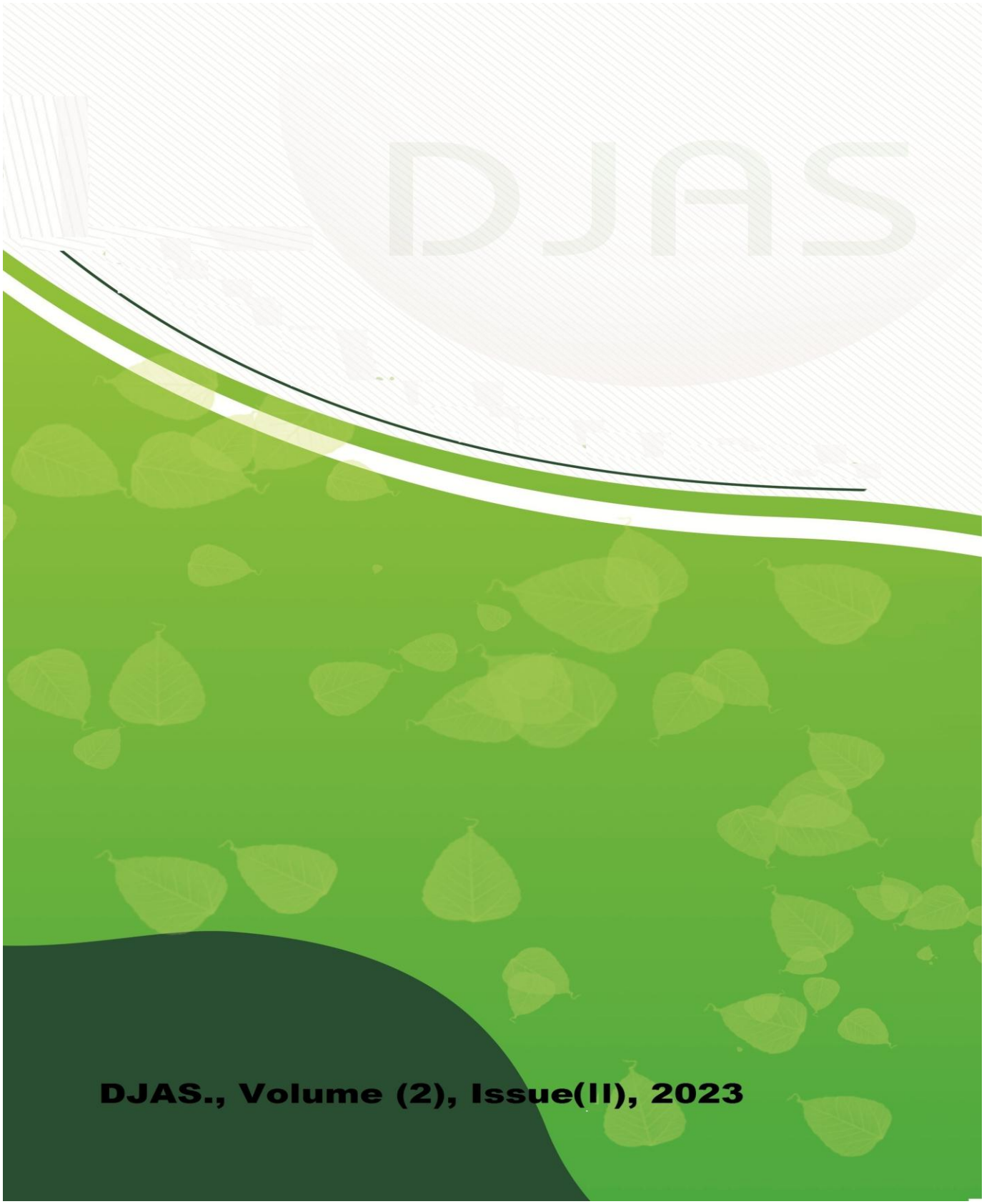
تم تقييم النشاط المضاد للبكتريا لمستخلصات الهكسان والميثان لكل من نبات الكزبرة والكمون ومعرفة التركيب الكميائى للمستخلص الأكثر فاعلية عن طريق تحليل كروماتوجرافيا الغاز- ومطياف الكتلة، حيث تم دراسة تأثير المستخلصات على أربعة أنواع من البكتيريا (الإستافيلوكوكس إيريس - الباسيلس سيريس - الإيشيريشيا كولاي – السيدوموناس إيروجينوزا). سجل أعلى قطر لمنطقة تثبيط على بكتيريا الإستافيلوكوكس إيريس وبكتيريا السيدوموناس إيروجينوزا ٣٣ مم و٣١.٥ مم على التوالي عند تركيز (١٠٠ ميكروليتر) لمستخلص الكزبرة الهكسانى كما سجل أعلى قطر لمنطقة تثبيط على بكتيريا الباسيلس سيريس والإيشيريشيا كولاي ٢٦.٦ مم و ٢٨ مم على التوالي عند تركيز (١٠٠ ميكروليتر) وذلك لمستخلص الكمون الميثانى وبدراسة هذا التأثير على البكتيريا باستخدام الميكروسكوب الإلكترونى الماسح أظهرت النتائج ضعف النمو وتثبيط الإنقسام وخلل في نظام تجميع الميكروبات وانكماش وتشوه بعض الخلايا الميكروبية. وبدراسة تأثير المستخلصات السابقة على فرق الجهد زيتا لبكتيريا الإستافيلوكوكس إيريس والباسيلس سيريس تبين انخفاض كبير في قيمة فرق الجهد زيتا نتيجة المعاملة بالمستخلصات حيث تغيرت من ٢٠.٩ ملى فولت إلى ٠.١٩٣ ملى فولت، -١٩.١ ملى فولت إلى ٠.٢٨٢ ملى فولت على التوالي. أظهرت نتائج تحليل مستخلص الكزبرة الهكسانى باستخدام كروماتوجرافيا الغاز- ومطياف الكتلة وجود سبعة عشر مركب أساسى أعلى قيمة كانت للمركب ١ و٣ و٥ سيكلوهبترين (١٩.٧١%)، وأظهرت نتائج تحليل المستخلص الميثانى للكمون اثنين وثلاثون مركب أساسى أعلى قيمة كانت للمركب ٤- بنزالديهايد، (١- ميثيل إثيل) (٢٥.٧٣%).

الكلمات المفتاحية: مضاد بكتيرى، الكزبرة، الكمون، كروموتجرافيا الغاز، فرق الجهد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