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Synergistic Effect of *Capparis Spinosa* Fruits Extract in Comparison with Ciprofloxacin Against Resistant *E. Coli* O157:H7

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Abstract:

This study was conducted to evaluate the synergistic effect between *Capparis Spinosa* fruits methanolic extract and ciprofloxacin against resistant *E. Coli* O157:H7, in varying concentration. This experiment was carried out through the ultrasonic alcoholic extraction of *C. Spinosa* fruits, and an extraction ratio of 24% was obtained. The extract showed pronounced concentration dependent antibacterial activity. The susceptibility study revealed that *E. Coli* O157:H7 was sensitive to *C. Spinosa* fruits. The findings of the present study indicate that the use of pronounced *C. Spinosa* fruits extract may have the perfect to be choice in clinical control. The concentration of *C. spinosa* fruits extract increase the activity of inhibition, increase as well when used in combination with ciprofloxacin (half concentration from each other). In result appeared synergism effect between plant extract and ciprofloxacin and may make bacteria lost their resistance to antibiotics by this synergism phenomenon**.** The result of MIC (6400 µg/ ml), MBC (12800 µg/ ml) and for *C.* spinose fruits extract while ciprofloxacin MIC (12.5 µg/ ml) and MBC (25 µg/ ml). MICs of CIP/ *C. spinosa* fruits extract combinations against *E. coli* O157:H7 isolate was 1.562/ 1600 µg/ml. Value of minimum bactericidal concentration (MBC) of the synergistic combinations (CIP/ *C. spinosa* fruits extract) was 3.124/ 3200 µg/ml. According to obtained results fractional inhibitory concentration (FIC) value of (CIP/ *C. spinosa* fruits extract 1.562/ 1600 µg/ml) was (0.375) less than 0.5 indicates synergistic effect of interaction.

Key words: *E. Coli* O157:H7, *C. spinose***,** Susceptibility test, synergistic, FIC**.**

Introduction:

E. coli O157:H7 is a member of a group of pathogenic *E. coli* (EHEC) that colonize the gastrointestinal tract and cause a condition known as hemorrhagic colitis (HC) or bloody diarrhea. Also a member of the larger category of Shiga toxin-producing *E. coli* (STEC). This group of *E. coli* is defined by its capacity to produce Shiga toxin type 1 (Stx1), Shiga toxin type 2 (Stx2), or both toxins **(1).** *E. coli* O157:H7 is an emerging bacterial zoonotic foodborne pathogen of global significance for which cattle is the primary reservoir **(2; 3).** That causes life-threatening infections such as bloody diarrhoea, abdominal pain, haemorrhagic colitis (HC), haemolytic uremic syndrome and kidney failure **(4).**

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Regarding of the continuing increase in the resistance of bacteria to antibiotics, the emergence of multi-resistant strains and the resulting therapeutic problems, as it is known that the synthetic drugs may cause a wide range of serious effects, thus use of herbal medicine is one of the promising solutions if it is based on scientific studies. Recently, several data are published in this direction, making it possible to value and rationalize the beneficial effect on health of medicinal plants **(5,6).** *C. spinosa* L. (Caper) is a perennial spiny shrub of Capparidaceae family, genus Capparis, is a glabrous, highly branched, spiny, spiked, relatively leafless bush or little tree developing fiercely in dry, open badlands all through the parched and semi-dry zones of diverse parts of the world **(7)**. Commonly known by different names such as Kabbar (Arab), Caper (English), câprier (French), Alcaparro (Spainish) **(8).** Ripened fruits of this plant have sharp hot taste; astringent to the entrails, decimates foul breath, biliousness and urinary purulent releases, it is useful in cardiovascular inconveniences **(9).** *C. spinoa* L. is an important medicinal plant **(10).** This plant is being utilized as a laxative, emmenagogue, alexipharmic, and aphrodisiac. It improves appetite and is good for rheumatism, cough, lumbago, hiccough, and asthma **(11**). In fact, *C. spinosa* roots leaves and fruits are traditionally used for the treatment of various diseases gastrointestinal disorders, skin diseases, earache, kidney and liver diseases **(12).**

Caper fruits were characterized to have the exocarp green in all stages of development, and there was a decrease in the protein content with the development of the fruit, while the fruits presented high contents of total phenols, flavonoids and flavanols **(11).** *Capparis* species are well known for their nutritional value and It possesses wide antimicrobial spectrum including antibacterial and antifungal activity in addition to their antioxidant, hepatoprotective, anticancer, antiallergic, anthelmintic, antidiabetic, anti-inflammatory, cytotoxic antiarthritic, anti-oxidant, cardiovascular, chondroprotective, anti-diabetic, hypolipidemic, antiallergic, anti-histaminic, immune modulatory, and anti-hepatotoxic activities and antihyperlipidemic along with their uses in the traditional medicine for controlling of many diseases. The leaves, roots and buds are used for treating gastric, earache, dermatological, liver and kidney disorders, while the fruits used for treating fever, diabetes, rheumatism and headache **(13-15)**.

Caper is a traditionally used medicinal plant and widely studied for its biological properties. Moroccan sample showed the highest phenolic content across all extraction types followed by Italian and Turkish **(16).** Roots were used as diuretic, astringent, and tonic. Bark root, which has a bitter taste, was used as appetizer, astringent, tonic, ant diarrheic and to treat hemorrhoids and spleen disease. Infusion of stems and root bark were used as anti-diarrheic and febrifuge. Fresh fruits were used in sciatica, and dropsy. Dried and powdered fruit combined with honey was used in colds, rheumatism, gout, sciatica and backache **(17).** A traditional Persian medicine formulation for diabetes mellitus are *C. spinosa*, with no notable hepatic, renal and gastrointestinal side effects **(18).** *C. spinosa* has several beneficial health effects on human diseases **(19).** The roots are used as astringent, appetizing, menstrual, tonic, and repellent to intestinal worms. It is also used for treating infections, and foreskin is used to treat rheumatism in the joints **(20)**.

Ciprofloxacin is a second-generation fluoroquinolone that has spawned many derivative antibiotics, active against several kinds of bacterial infections, many gram negative and gram-positive bacteria **(21; 22)**. There is no cross resistance between fluoroquinolones and other classes of antibiotics, so it may be of clinical value when other antibiotics are no longer effective **(23).** Ciprofloxacin is a generic drug. It is a very popular fluoroquinolone that can treat a number of bacterial infections. The quinolones are family or a group that has activity against both - negative and positive –gram bacteria, when compared to Ciprofloxacin **(24).** Ciprofloxacin acts on

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bacterial topoisomerase II (DNA gyrase) and topoisomerase IV **(25).** The mechanism of action of ciprofloxacin antibiotics is inhibition of DNA gyrase and partly of bacterial topoisomerase IV, resulting in cell death due to complex binary or tertiary formations among DNA, the enzyme and the antibiotic, Ciprofloxacin have bactericidal activity and the mechanisms of bacterial resistance are primarily linked to modifications at the binding site or active drug extrusion from the bacteria through efflux pumps **(26).**

Adverse effects are mild at therapeutic doses and are mostly limited to gastrointestinal disruptions such as nausea and diarrhea. The serious adverse effects of ciprofloxacin include peripheral neuropathy, prolonged QT interval, seizures, and other CNS effects, hyper or hypoglycemia, photosensitivity, tendonitis. Black box warnings include tendinitis and tendon rupture (associated with the fluoroquinolone class). The most common type of tendon rupture involves the Achilles tendon. There have also been reports of tendinopathies in the gluteal, iliopsoas, and triceps tendons **(27; 28).** Rare interactions include drug-induced bullous pemphigoid **(29)**. The use of quinolones has been associated with the development of serious and persistent adverse drug reaction (ADR) mainly affecting muscles, joints and the nervous system. This risk has led the European Medicines Agency (EMA) to endorse some restrictions on the use of this class of antibiotic **(30)**. Ciprofloxacin overdose typically leads to acute renal failure**.** An overdose may progress over the next 6 days with rising serum creatinine and BUN, as well as anuria**,** Patients may require prednisone therapy, urgent hemodialysis, or supportive therapy, depending on the degree of overdose, patients may recover normal kidney function or progress to chronic kidney failure **(31).**

This study Aims to evaluate the synergistic effect between *C. spinosa* fruits methanolic extract and ciprofloxacin against resistant *E. Coli* O157:H7.

Materials and Methods:

Collection of *Capparis Spniosa* Fruits

Fresh *C. spinosa* fruits were collected from different region in Baghdad. Fruits was washed thoroughly in water, cut into small pieces and dried in shade at 25°C until a constant weight was reached. Dried fruits were pulverized by grinder and particles with size distribution of less than 40-mesh was used for the extraction. It was stored at 4°C until use for extraction.

Extraction of *Capparis spinosa* Fruits (Ultrasound- Assisted Extraction)

C. spinosa fruits powder (10g) and 100 ml of methanolic solvent are introduced into a flask. The mixture was exposed to bath ultrasound for 1 hour under 60 kHz at room temperature and sheltered from light. The later process has been repeated under same condition for ten times. Afterward, the mixture was filtrated and the final volume (accumulate filtrate) is concentrated in rotary evaporator under reduced pressure **(32)**, extract was stored at -18 °C until the tests **(33).**

In *Vitro* Antibacterial Activity of Ciprofloxacin, *Capparis Spinosa* Fruits Methanolic Extract and Combination of Ciprofloxacin/*C. Spinosa* Fruits Methanolic Extract Against *E. Coli* O157:h7:

Source of Bacteria, Activation and Identification:

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The strain of *E. coli* O157: H7 bacteria was obtained from the laboratories of Al-Karama Hospital in Wasit Governorate, isolated and diagnosed, Bacterial isolate was activated then it was be identified by studying morphological and biochemical characteristics **(34).** Gram stain is an important procedure that has been used to identify the phenotypic description of bacteria. Morphological colonies characterization was recorded on the media by using (MacConkey agar and eosin methylene blue (EMB) agar, sorbitol MacConkey agar and chrome agar *E. coli* O156: H7 tested their shape, size, color for primary identification of *E. coli*. and it was diagnosed as *E. coli* O157: H7 bacteria by biochemical test as the following: Indole, Catalase Test, Coagulase Test, oxidase test, motility test, methyl red test and voges-proskauer and by vitek 2 system.

Sensitivity Test:

The agar well diffusion method was adopted according to **(35),** for assessing the antibacterial activity of ciprofloxacin, *C. spinosa* fruits methanolic extract and combination of ciprofloxacin/ *C. spinosa* fruits extract. Five ml of standardized bacterial stock suspension (1.5×10^8 cfu/ml) of *E. coli* O157: H7 was mixed with 500 ml of sterile Mueller Hinton agar, then 25 ml of the inoculated Mueller Hinton agar was distributed into sterile petri dishes of each. The agar was left to set for 10 minutes to allow solidifying, then a 4 wells 6 mm in diameter were made using a sterile Pasteur pipette. After that, each wells were filled with 100 microliters containing different concentration from ciprofloxacin, *C. spinosa* fruits extract and combination (ciprofloxacin/ *C. spinosa* fruits extract) and last filled with distal water as control, which allowed to diffuse at room temperature for two hours. The plates were incubated at 37°C for 24 hours and five replicates were carried out for each concentration of antibacterial agent. The antibacterial activity was determined by measuring the diameter of inhibition zone around each well by millimeter against *E. coli* O157: H7.

C. spinosa fruits extract activity by using the concentrations of (400, 800, 1600, 3200, 6400, 12800 and 25600 µg/ ml) while Ciprofloxacin concentrations of (0.390, 0.781, 1.562, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 and 800 µg/ ml), combination activity (*C. spinosa* fruits extract/ ciprofloxacin) by using the concentrations of (0.195/200, 0.390/400, 0.781/800, 1.562/1600, 3.12/3200, 6.25/6400 and 12.25/12800 µg/ ml)**.**

Pharmacodynamics Analysis of *C. Spinosa* Fruits Methanolic Extract in Comparison with Ciprofloxacin.
Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Ciprofloxacin, and *C. spinosa* fruits extract:

For this purpose, a stock solution of ciprofloxacin, and *C. spinosa* fruits extract and combination of ciprofloxacin/ *C. spinosa* fruits extract were prepared in Mueller-Hinton broth, then make series dilution in different concentration that ranges between (0.390 to 50 µg/ ml and 400 to 25600 µg/ ml) of ciprofloxacin, *C. spinosa* fruits extract respectively were prepared in 96 well micro-titer plate, each well was inoculated with 100 µl of 10^6 CFU/ml *E. coli* O157:H7 and incubated on 37 ºC for 24 hrs. **(36)**, For colorimetric identification of bacterial growth, adding 20µl of TTC indicator (0.125% w/v) to each well of the test and re-incubated for two hrs.

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(36).

Determination of Minimum Inhibitory Concentration (MICs), MBCs for combinations of ciprofloxacin and *C. spinosa* fruits extract

The bacterial isolate (*E. coli*) was tested against combinations of ciprofloxacin and *C. spinosa* fruits extract. According to **Veiga *et al.*, (37)** the microdilution tests were performed in sterile a stock solution drug and extract were made in Mueller- Hinton broth, Briefly, the final concentration of CIP, and *C. spinosa* fruits extract in 200 µl MHB were ranges between (0.195-25 µg/ ml) and (200-25600 µg/ ml) respectively, from this broth, two folds dilution was downgraded from 100 µg in U- shape (400 µl well capacity) 96 well micro-titer plate, Each well was inoculated with 100 µl of (10^6 CFU/ml of *E. coli* O157:H7) The wells containing 200 µl of un-inoculated and inoculated MHB were considered as negative and positive controls, respectively and incubated at 35 ºC for 22 hrs. . For colorimetric identification of bacterial growth, 20µl of 0.125% TTC dye was added to each well were incubated again for 2 h.

To Estimation the effect of combination of antibiotics and extract, the fractional inhibitory concentration (FIC) index was done. Interpretation of FICIs was as follows: **(38).**

The interpretation of the results is done by calculating the fractional inhibitory concentration index ICFI:

FICI = ΣFIC = FIC A + FIC B

Where, FIC A is the MIC of extract in the combination / MIC of extract alone, and CFI is the MIC of antibiotic in the combination / MIC of antibiotic alone ; So reading the results will be taken into account the values found of the FICI.

FICI ≤ 0,5: Synergistic effect; 0,5 <FICI <2: Indifferent effect; FICI ≥2: Antagonistic effect.

The fractional inhibitory concentration index (FICI) for combination of ciprofloxacin and *C. spinosa* fruits methanolic extract was calculated as follows:

$$FICI_{A/B} = \frac{\text{MICA (combination)}}{\text{MICA (Alone)}} + \frac{MICB\ (combination)}{\text{MICB (Alone)}}$$

Result And Discussion:

Extraction by methanol (absolute 99.8%) and Phytochemical Analysis of *C. spinosa* Fruits. Extraction

In this study, the extraction ratio of *C. spinosa* fruits powder was 24 %, This result was identical with the results obtained by **(39)** which yield 29.5% from the shade-dried fruit, of *C. spinosa*, the differences in the amount of extract may be attributed to differences in the apparatus of extraction percolation method while in this study used ultrasonic water path extraction. The color of the extract was deep brown and the texture was semi gelatinous and sticky.

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Bacterial Identification:

E. coli O157:H7 appeared as gram negative, pleomorphic rods and non-spore forming under light microscope. The biochemical tests results for bacterial isolate are positive for catalase, Indole and Methyl Red test (MR), while they were negative for Voges – Proskaure (VP), Citrate, and oxidase test.

MacConkey agar is an indicator, selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric bacilli that distinguish them on the basis of lactose fermentation with the presence of crystal violet and bile salts which inhibits the growth of Gram-positive species, that facilitates the selection and isolation of gram-negative bacteria. Bacteria that ferment lactose, such as *E. coli* can produce acid that reduces the pH of the agar below 6.8 and results in a pink colonial appearance **(40).** The result showed gram negative lactose fermenter *E. coli* bacterium. *E. coli* O157: H7 on Eosin Methylene Blue (EMB) agar show colonies as green metallic sheen with a dark center, EMB agar also commonly used as both a selective and a differential medium for Gram-negative bacteria against Gram-positive bacteria. EMB agar Using in the insulation of *E coli* after enrichment is due to the fact that it gives a specific metallic sheen color on this medium which is characteristic of all *E. coli* serotypes as well as media designed to inhibit the growth of gram-positive bacteria. *E. coli* gave metallic sheen color on EMB agar because this media contains both eosin and methylene blue dyes which have metachromatic properties so the fast lactose fermenters *E. coli* contain acid that reduces the pH, facilitates the colonizing and provides it purple color – black color that makes the metallic characteristic shine when exposed to light **(41).**

Colonies with metallic sheen on EMB agar which is a typical feature of *E. coli* was transferred to sorbitol MacConkey agar to check for the presence of *E. coli* O157 phenotype **(42).** Growth findings on Sorbitol MacConkey agar appeared as colorless or amber-like colonies called non sorbitol fermenting isolates (NSF), *E. coli* O157:H7 strain could be detected by differential growth on sorbitol MacConkey agar (SMAC), because O157:H7 strain do not ferment sorbitol **(43).** This medium is recommended for *E. coli* O157:H7 isolation, fermenting lactose but not fermenting sorbitol, and therefore producing colorless colonies. *E. coli* O157:H7 differs from most other strains of *E. coli* in being unable to ferment sorbitol. In Sorbitol MacConkey agar, lactose replaced by sorbitol, most strains of *E. coli* ferment sorbitol to produced acid, but *E. coli* O157:H7 cannot ferment sorbitol, so can differentiate it from other strains of *E. coli* depending on the fact that *E. coli* O157:H7 unlike 90% of *E. coli* isolates does not ferment sorbitol **(44).** *E. coli* O157:H7 colonies were appeared as mauve color after culturing on Chrome agar.

These results showed that Chrome agar aids in diagnosis of *E. coli* O157:H7, *E. coli* O157:H7 utilized one of chromogenic substrates which produced mauve colored colonies, this was in agreement with **Yousif and al-Taii, (45)** and **Al Dawmy and Yousif, (46)** who reported that the Chrom agar O157 was useful for diagnosis of *E. coli* 0157: H7. The use of chrom agar O157:H7 in the present study was used for the identification of O157:H7 strain, in which O157:H7 strain shows mauve color colonies, while non O157 appears either blue, white or inhibited, such color variability arises because this medium contains a specific mixture of artificial chromogenic conjugates consisting of an *E. coli*-specific enzyme substrate coupled with a chromophore. The colorless conjugation was released in the cleaves of *E. coli*, which give the *E. coli* colonies a distinctive color **(47).**

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Identification of *E. coli* O157:H7 by VITEK® 2 System:

Confirmation of identification of *E. coli* O157:H7 was performed with the automated Vitek 2 system by using GN-ID cards which contain (64) biochemical tests, the isolate bacteria have been achieved an excellent identification level with a probability of 98% based on the manufacturers technical datasheet. The results demonstrate that *E. coli* O157:H7 were confirmed with ID message confidence level ranging excellent (Probability percentage from 93 to 98%). This technique is characterized by fast detection of bacteria without need for many of culture media as well as reduces cultures contamination **(48).** Automated bacterial identification in the clinical laboratory provides a rapid and reliable diagnosis for most pathogens involved in infectious diseases with a highly acceptable level of identification accuracy **(49). Al Humam, (50)** found the benefit of using Vitek2 system for comparing biochemical characteristics of *E. coli.*

***In-vitro* Antibacterial Activity of *Capparis Spinosa* Fruits Extract:**

The size of inhibition zones was different according to concentration of *C. spinosa* fruits extract, the size of inhibition zone was proportionally increased with increasing of concentration of *C. spinosa* fruits extract (table 1). The results showed that *E.* O157: H7 was sensitive to all concentrations using in this study. In all used concentration there was a significant increase ($P<0.05$) in diameter of zone of inhibition in *E. coli* O157:H7. Distilled water was used as control, it did not give any noticed zone of inhibition, and distilled water was used as a solvent for *C. spinosa* fruits extract through *in-vitro* studies.

Table (1): Antibacterial activity *in vitro* (zone of inhibition (mm.) for different concentrations of *C. spinosa* fruits extract against *E.* O157: H7 compared with distilled water.

Conc. µg/ ml / Zone of inhibition mm.	400	800	1600	3200	6400	12800	25600	LSD value
***C. spinosa* fruits extract**	14.0 ±0.31 A g	16.0 ±0.71 A f	18.0 ±0.31 A e	21.0 ±0.94 A d	23.0 ±0.83 A c	25.0 ±0.54 A b	28.0 ±0.71 A a	1.92 *
Distilled water	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 NS
LSD value	0.729 *	1.63 *	0.729 *	2.18 *	1.92 *	1.26 *	1.63 *	---
Means with different big letters in the same column and small letters in the same row are significantly different. * ($P\leq0.05$).								

The results of this study agreement with **AL-Azawi *et al.*, (51)** who attributed that the aerial parts of *C. spinosa* consider as a potential source of antibacterial compounds and the extracts of *C. spinosa* parts were reported to be effective to inhibit the growth of different bacterial strains especially those which have acquired resistance to antibiotics. **Hamad *et al.*, (52)** reported that the *C. spinosa* inhibits the growth of the isolates of *E. coli* that cause disease.

Oudah *et al.*, (53) reported the highest activity of extracted polyphenolic for *C. spinosa* against *E. coli* and showed 12mm inhibition zone. **Hameed *et al.*, (54)** noticed that the majority of phenols, regardless of their source from the plant, have shown efficacy against bacteria, and this indicates an increase in the concentration of the active components in the reproductive parts

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represented by the flower and the fruits, to which the medicinal or physiological effect of the plant and its medicinal value are attributed, *C. spinosa* are one of the richest herbs with active ingredients, because of the diversity in the tremendous chemicals found in plants. **Al-Akedi *et al.*, (55)** reported that the Gram-positive bacteria are resistant to these extracts, while negative bacteria were variable in their effect according to the type of germ and the part of the *C. spinosa* plant used. The aqueous and alcoholic extracts of the fruit were most likely to inhibit gram-negative bacteria. **Hameed *et al.*, (54)** reported that the Phenols in *C. spinosa* fruits have the highest inhibitory value for tested bacteria *Escherichia coli*. The alcoholic fruit extract was the most effective in comparison the aqueous extract may be due to the effectiveness of the alcoholic extract in inhibiting bacteria or to the active compounds may be soluble in organic solvents **(56). Ennacerie *et al.*, (6)** reported that the various extracts from *C. spinosa* (fruits or flower buds) tested have an interesting antibacterial activity *in vitro* and the two types of the extracts of the flower buds and the fruits have a power of inhibition of the growth of the pathogenic germs of the same efficiency on the Gram-positive and the Gram-negative and reported the calculation of the MBC / MIC, which informs about the bactericidal effect of the extract, confirms that the alcoholic extract of the fruits generally has a lethal effect.

***In Vitro* Antibacterial Activity of Ciprofloxacin Against *E. coli* O157:H7.**

Different concentrations of ciprofloxacin (1.562, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 and 800 µg/ml) were used in agar well diffusion assay, caused different degrees of zones of inhibition against *E. coli*. O157:H7 The size of inhibition zones was different according to concentration of ciprofloxacin, the size of inhibition zone was proportionally increased with increasing of concentration of ciprofloxacin (table 2). The results showed that *E. coli* O157:H7 resistant to ciprofloxacin. In all used concentration there was a significant increase ($P<0.05$) in diameter of zone of inhibition in *E. coli* O157:H7. Distal water was used as control, it did not give any noticed zone of inhibition, and distilled water was used as a solvent for ciprofloxacin through *in-vitro* studies.

Table (2): Antibacterial activity *in vitro* (zone of inhibition (mm.)) for different concentrations of ciprofloxacin against *E.coli* O157:H7 compared with distilled water.

Conc. µg/ ml / Zone of inhibition mm	6.25	12.5	25	50	100	200	400	800	LSD value
Ciprofloxacin	8.0± 0.55 A h	10.0± 0.31 A g	12.0 ±0.54 A f	14.0 ±0.31 A e	16.0 ±0.54 A d	18.0 ±0.71 A c	21.0 ±0.54 A b	24.0 ±0.31 A a	1.34 *
Distilled water	0.00 ±0.00 B a	0.00 ±0.00 B a	0.00 ±0.00 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.00 B a	0.00 ±0.00 B a	0.00 ±0.00 B a	0.00 NS
LSD value	1.26 *	0.729 *	1.26 *	0.729 *	1.26 *	1.63 *	1.26 *	0.729 *	---

***In-vitro* Antibacterial Activity of Combination (Ciprofloxacin + *Capparis Spinosa* Fruits Extract):**

The antibacterial properties of combination (ciprofloxacin + *C. Spinosa* Fruits extract) by using the agar well diffusion assay against *E. coli* O157: H7. By using half concentration from each antibacterial agent (ciprofloxacin + *C. spinosa* fruits extract) they demonstrated that the combination produced inhibition zone sizes between 18-32 mm. The size of inhibition zones was different

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according to concentration of combination, the size of inhibition zone was proportionally increased with increasing of concentration of combination (table 3). The results showed that *E. coli* O157: H7 was sensitive to all the concentrations. In all used concentration there was a significant increase (P<0.05) in diameter zone of inhibition in *E. coli* O157:H7. Distilled water was used as control, it did not give any noticed zone of inhibition.

Table (3): Antibacterial activity *in vitro* (zone of inhibition (mm.) for different concentrations of combination (ciprofloxacin+ *C. spinosa* fruits extract) against *E. coli* O157:H7) compared with distilled water.

Conc. µg/ ml / Zone of inhibition mm	0.195 + 200	0.390 + 400	0.781 + 800	1.562 + 1600	3.12 + 3200	6.25 + 6400	12.25 + 12800	LSD value
Combination	18.0 ±0.94 A f	20.0 ±0.70 A ef	22.0 ±1.14 A de	24.0 ±0.94 A cd	26.0 ±0.54 A c	29.0 ±0.94 A b	32.0 ±0.71 A a	2.47 *
Distilled water	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 ±0.0 B a	0.00 NS
LSD value	2.18 *	1.63 *	2.62 *	1.93 *	1.26 *	2.18 *	1.63 *	---

Pharmacodynamics Analysis of Ciprofloxacin and *Capparis Spinosa* Fruits Extract:

Minimum Inhibitory Concentration, Minimum Bactericidal Concentration:

The findings showed that the concentration 12.5 µg/ml of ciprofloxacin was active against *E. coli O157:H7* isolate. The tested in micro-dilution assay as shown in the (figure.1). Similarly, the same bacteria (*E. coli* O157:H7*)* was inhibited at the concentration of 6400 µg/ml of *C. spinosa* fruits extract. *E. coli* O157:H7 was recorded MIC value (12.5 and 6400 µg/ml) of ciprofloxacin and *C. spinosa* fruits extract respectively. This isolate of *E. coli O157:H7* was recorded MBC value (25 and 12800 µg/ml) of ciprofloxacin and *C. spinosa* fruits extract respectively. Based on visual readings **Veiga *et al.* (37)** performed by watching the development or not of red color originating from the reductions of TTC (colorless) to formazan (red). The lowest concentration was regarded and no color development occurred to determine the MIC and MBC, confirming the results shown in the experiment. Many methods were used to determine the MIC, but the micro dilution assay is the most adopted and accredited method by the European Committee on antimicrobial susceptibility testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) to determine the MIC **(57).**

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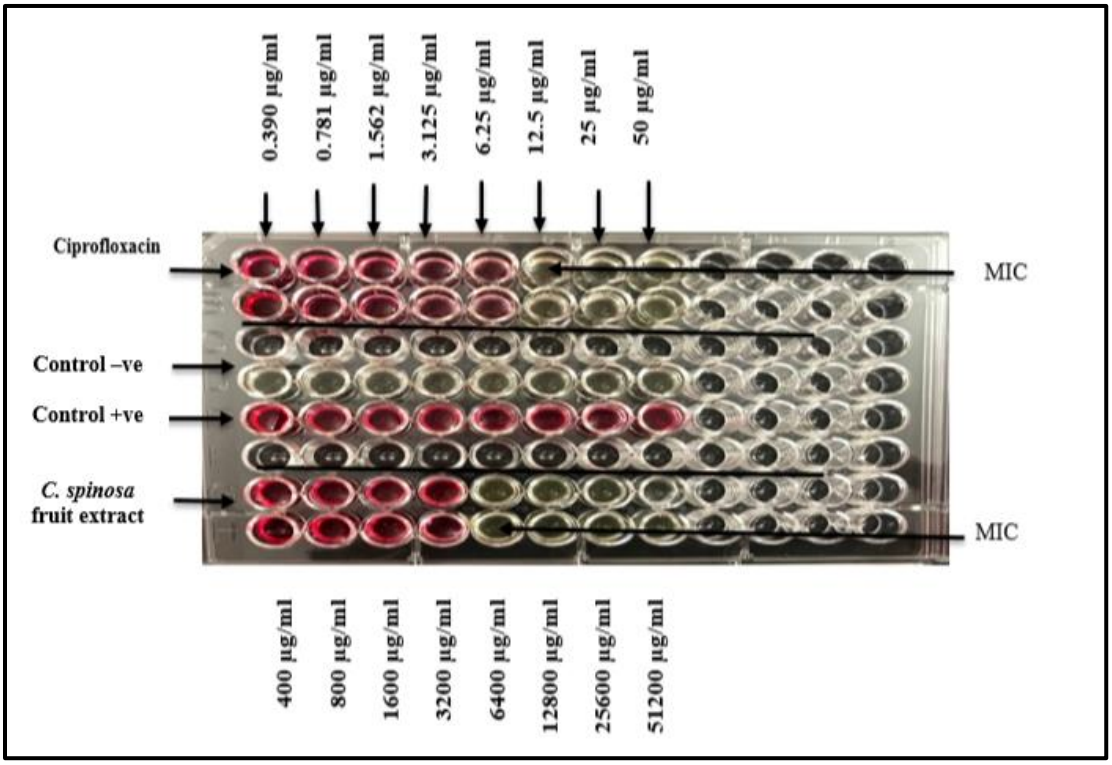

Figure (1): Minimum inhibitory concentration of Ciprofloxacin and *C. Spinosa* Fruits Extract against *E. coli O157:H7* Isolates, White (No growth) pink (growth).

Pharmacodynamics Analysis of Combination (Ciprofloxacin + *C. Spinosa* Fruits Extract) Against *E. coli* O157:H7.

Minimum Inhibitory Concentration (MICs) and Minimum bactericidal Concentration (MBC)

The results showed that the MICs of CIP/ *C. spinosa* fruits extract combinations against *E. coli* O157:H7 isolate was 1.562/ 1600 µg/ml. The checkerboard assays showed, the minimum inhibitory concentration (MICs) of these agents were decreased significantly in combinations and exhibited a synergistic effect (FIC index < 0.5) against *E. coli* O157:H7.

According to obtained results in figure (2), fractional inhibitory concentration (FIC) value of (CIP/ *C. spinosa* fruits extract 1.562/ 1600 µg/ml) was (0.375) less than 0.5 indicates synergistic effect of interaction. Value of minimum bactericidal concentration (MBC) of the synergistic combinations (CIP/ *C. spinosa* fruits extract) was 3.124/ 3200 µg/ml. The efficiency of combination of the ciprofloxacin and *C. spinosa* fruits extract against resistant *E. coli* O157:H7 was assessed using the checkerboard method. The synergistic effect (FIC index < 0.5). **Al-Akedi *et al.* (55)** reported The aqueous and alcoholic extracts of plant's fruit appear to be most effective. As the concentration of plant extracts increase the activity of inhibition increase as well. In some cases, plant extract activity was more effective than studied antibiotics and also result showed synergism effect between plant extract and antibiotics and some studied bacteria lost their resistance to antibiotics by this synergism phenomenon**. Al-Akedi *et al.*, (55)** noted through the results of the experiments of the synergistic action of the extract of the fruit of the *C. spinosa* Aqueous with a number of antibiotics,

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and the presence of the extract with the antibiotic In general, it had a positive effect in increasing the inhibitory activity against the studied bacteria, It is noted that of *E.coli.* was the effect of the antibiotic and the extract together result inhibition diameters higher than the presence of the antibiotic alone for each antibiotic, in diameter zone for S, CIP, and CTX (18, 35, 30,) mm, respectively. Compared to using the antidote alone.

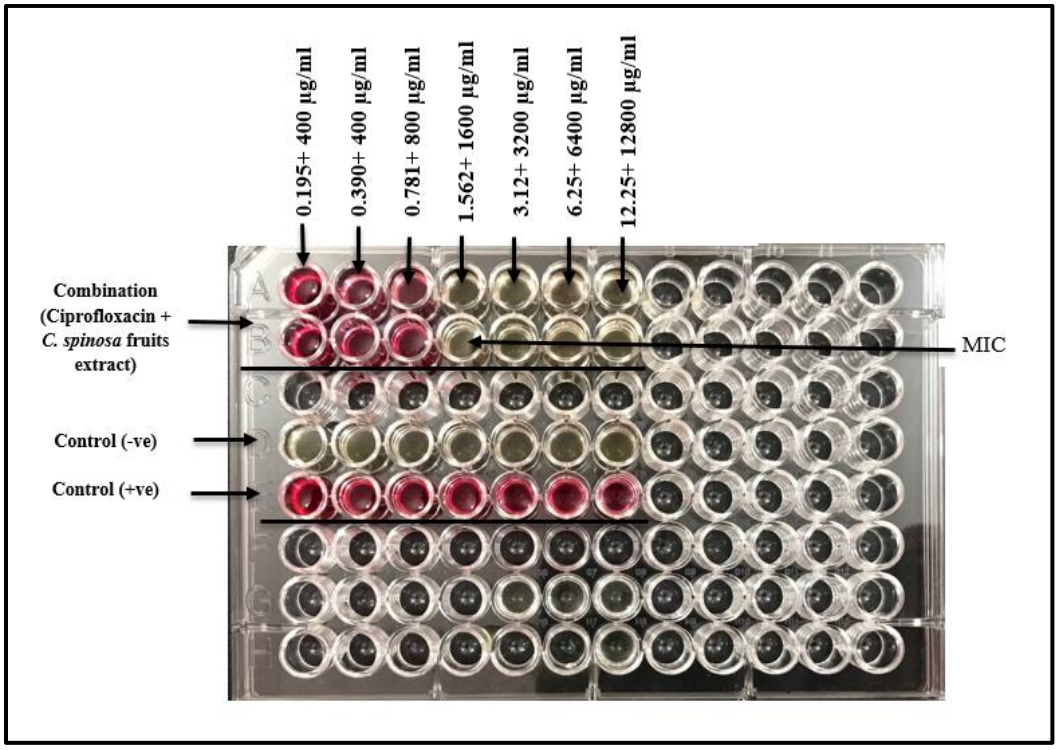

Figure (2) Checkerboard assay of ciprofloxacin and *C. spinosa* fruits extract combination.

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