Evaluation the Activity of Capparis Spinosa Fruits Extract Against Resistant E. Coli O157:H7

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Abstract
This study was conducted to evaluate the antibacterial activity of Capparis Spinosa fruits methanolic extract against resistant E. Coli O157:H7 in comparison with ciprofloxacin, 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 result of MIC (6400 µg/ ml), MBC (12800 µg/ ml) for C. spinose fruits extract while ciprofloxacin MIC (12.5 µg/ ml) and MBC (25 µg/ ml). Mutant preventive concentration of ciprofloxacin and C. spinosa fruits extract against E. coli O157:H7 (MPC/MIC) = 2.
Keywords: C. spinose, E. Coli O157:H7, susceptibility test, methanolic, extract.

1. 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). That causes life-threatening infections such as bloody diarrhoea, abdominal pain, haemorrhagic colitis (HC), haemolytic uremic syndrome and kidney failure (3). 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 (4,5).
Capparis 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 (6). Commonly known by different names such as Kabbar (Arab), Caper (English), câprier (French), Alcaparro (Spainish) (7). C. spinosa plant is a thorny, and has deep roots, which can extend up to 6-10 m (8,9). Ripened fruits of this plant have sharp hot taste; astringent to the entrails, decimates foul breath, biliousness and urinary purulent releases (10). C. spinoa L. is an important medicinal plant and being utilized as a laxative, emmenagogue, alexipharmic, and aphrodisiac. It improves appetite and is good for rheumatism, cough, lumbago, hiccough, and asthma ((11,12). 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 (13). Pharmacological effects of C. spinosa have reported a wide range of biological activities including antioxidant, anti-inflammatory, anti-bacterial, anti-parasitic, anticancer, immunomodulatory, antiviral, antiplaque, anticarcinogenic, antihepatotoxic, antimicrobial, and antidiabetic effects (14-16).
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 (12).
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 (17-19).
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 (20). The whole plant was used for

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rheumatism. 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 (16).

A traditional Persian medicine formulation for diabetes mellitus are c. spinosa, with no notable hepatic, renal and gastrointestinal side effects (21). c. spinosa has several beneficial health effects on human diseases (22). The antioxidant, nephroprotective and hepatoprotective effects of methanolic extract of its leaves associated with its phytochemical content, and nine compounds namely rutin, resveratrol, coumarin, epicatechin, luteolin, catechin, kaempferol, vanillic acid and gallic acid are more responsible in support of traditional usage of C. spinosa to cure kidney and liver diseases (23). 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 (24). The antioxidant activity of various parts of caper was reported from different scholars (25).

This study Aims to evaluate antibacterial activity of C. Spinosa fruits extract against resistant E. coli O157:h7.

2. 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 (26), extract was stored at -18 ºC until the tests (27).

In vitro antibacterial activity of Capparis spinosa Fruits Methanolic Extract Against E. Coli O157:H7.

Source of Bacteria, Activation and Identification

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 **(28).** 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 (29), for assessing the antibacterial activity of the prepared C. spinosa fruits methanolic extract and standard antibacterial ciprofloxacin. Five ml of standardized bacterial stock suspension (1.5 × 108 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 C. spinosa fruits extract, ciprofloxacin and last filled with distal water, 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. The antibacterial activity was determined by measuring the diameter of inhibition zone around each well by millimeter against the tested bacteria.

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/100 ml). 0.1 ml of sterilized distilled water was served as a control.

Pharmacodynamics Analysis of *C. Spinosa* Fruits Methanolic Extract in Comparison with Ciprofloxacin.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration:

For this purpose, a stock solution of ciprofloxacin, and c. spinosa fruits extract were prepared in Mueller-Hinton broth, then make series dilution in different concentration that ranges between (0.390_ 50 µg/ ml) of ciprofloaxacin were prepared, and range between (400_ 25600 µg/ ml) in 96 well micro-titer plate, each well was inoculated with 100 µl of 106 CFU/ml E. coli O157:H7 and incubated on 37 °C for 24 hrs. (30), 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. (31).

Mutant Prevention Concentration

An amount of 0.1 ml of freshly prepared 1.5 x 108 CFU/ml (equivalent to 0.5MacFarland) E. coli suspension was transferred to 14.9 ml of trypticase soy broth to obtain 106 CFU/ml bacterial suspension then incubated on 37 °C for 2 hrs. to bring E. coli to the log phase of bacterial growth. Plates of Mueller-Hinton agar and ciprofloxacin or c. spinosa fruits extract had been prepared and a calculated aliquot was diluted in previously prepared Mueller-Hinton agar (45-50 °C) to produce 4x, 2x, 1x, 0.5x and 0.25x MICs concentrations. Each concentration for ciprofloxacin and also for C. spinosa fruits extract was poured into a petri dish (triplicate) and 0.1 ml of bacterial suspension was spread on each plate then incubated at 37 °C for 72 hrs. The lowest antibiotic concentration or lowest extract concentrations that recorded no visible growth of bacteria is considered the concentration that prevents bacterial mutations (32).

3. Results and Discussion

Extraction by Methanol (absolute 99.8%) of *Capparis spinosa* Fruits.

In this study, the extraction ratio of C. spinosa fruits powder was 24 %, This result was identical with the results obtained by (33) 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.

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, oxidase and coagulase 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 (34). 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 (35).

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 (36). 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 (37). 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 (38). 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 (39) and Al Dawmy and Yousif (40) 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 (41).

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 (42). 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 (43). Al Humam (44) 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 extract against bacteria 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	0.00 ±0.0 B	0.00 ±0.0 B	0.00 ±0.0 B	0.00 ±0.0 B	0.00 ±0.0 B	0.00 ±0.0 B	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≤0.05).

The results of this study agreement with AL-Azawi et al., (45) 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., (46) reported that the C. spinosa inhibits the growth of the isolates of E. coli that cause disease.

Oudah et al., (47) reported the highest activity of extracted polyphenolic for C. spinosa against E. coli and showed 12mm inhibition zone. (Hameed et al., (48) 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 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.

Mohsen et al., (49) 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., (48) 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 (50). Ennacerie et al., (5) 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 resistance to ciprofloxacin. In all used concentration there was a significant increase (P<0.05) in diameter of zone of inhibition in *E. coli*'. 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± 0.55A 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 *	---

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. (31) 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 (51).

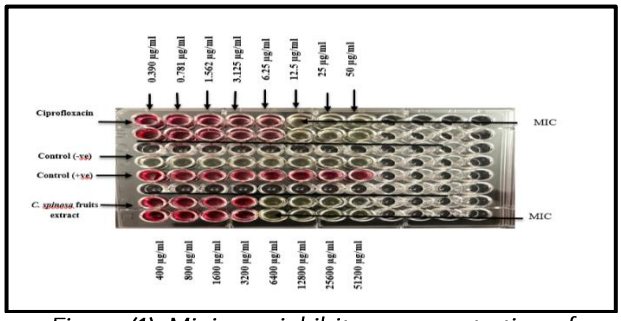

Figure (1): Minimum inhibitory concentration of Ciprofloxacin and C. Spinosa Fruits Extract against E. coli O157:H7 Isolates, White (No growth) pink (growth).

Mutant Prevention Concentration

Based on the recorded MICs (12.5 and 6400 µg/ ml) of ciprofloxacin and C. spinosa fruits extract respectively against E. coli O157:H7, the observations of our mutant prevention concentration (MPC) after 72 hrs. of incubation were reported in the Table (3). The results revealed that there was no visible bacterial growth on the plates that contain 4x MIC, and 2x MIC of ciprofloxacin and C. spinosa fruits extract in comparison to the control plate. The results of MPC for 1x MIC concentration of ciprofloxacin and C. spinosa fruits extract showed a visible weak growth of E. coli O157:H7 in comparison to the control plate. Also, there was a heavier bacterial growth was appeared in the plate that contains 0.5x MIC concentration of ciprofloxacin and C. spinosa fruits extract in comparison to the plate that contains 1x MIC concentration. The Mutation prevention index (MPC/MIC) which calculated depending on the recorded results of mutant prevention concentration to the minimum inhibitory concentration of the used isolate of E. coli O157:H7 in the test of ciprofloxacin and C. spinosa fruits extract was two. Adwan and Omar, (52). showed that aqueous leaf extract of C. spinosa possesses genotoxic and mutagenic potential effects on E. coli. In addition, the results also point out the capability of using C. spinosa to treat and prevent infections caused by several microorganisms, further studies are recommended to determine the specific ingredients in this plant as well as the correct mechanisms responsible for that genotoxicity.

Table (3): Mutant preventive concentration of ciprofloxacin and *C. spinosa* fruits extract against *E. coli* O157:H7.

Ciprofloxacin, *C. spinosa* fruits extract							
Concentration	control	0.25 MIC	0.5 MIC	1 MIC	2 MIC	4 MIC	MPC/MIC
Observation	+	+	+	+	-	-	2

(+) Visible bacterial growth, (-) No bacterial growth.

Results that is reported in the table (3) revealed that the lowest concentration of ciprofloxacin and c. spinosa fruits extract that prevents bacterial mutation were 25 and 12800 µg/ml (2x MIC) respectively; this value is equivalent to the minimum bactericidal concentration obtained from the time killing curve of ciprofloxacin and c. spinosa fruits extract. Mutant prevention concentration (MPC) is the concentration of the antibacterial agent that prevents heavy bacterial inoculum (> 109 CFU/ml) from visible growth on the plate (53). The main difference between MIC and MPC is the used bacterial inoculum since the standard bacterial inoculum used in MIC (106 CFU/ml) is less dense than the inoculum that used in MPC (> 109 CFU/ml) because the 1st bacterial mutation might occur over 109 CFU/ml bacterial population; with other words, MPC will deprive the bacterial chance to mutate or to develop resistance (54). The ratio of (MPC/MIC) is another calculated parameter and it is defined as "The MPC is the highest value in the concentration range whereas the MIC is the lower value". Susceptible bacteria's growth is inhibited within this range, yet resistant mutant subpopulations can still be selectively amplified. Such Low value of the MPC/MIC will show that we are more capable of preventing the creation of mutations because it will narrow the gap between the MPC and MIC, whereas the high MPC/MIC ratio will give chance for more mutations (55).

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