THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Effect of Ultrasonic Extract of *Capparis spinosa* Fruits Against *E. coli O157:H7*

Reham N Abdulridha , Ali H Saliem*

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

***Correspondence**:
ali.h@covm.uobaghdad.edu.iq

Received: 20 May 2023
Revised: 06 Jun 2023
Accepted: 15 Jun 2023
Published: 28 Jun 2023

DOI:
https://doi.org/10.30539/ijvm.v47i1.1529

Cite:
Abdulridha RN, Saliem AH. Effect of ultrasonic extract of *Capparis spinosa* fruits against *E. coli* O157:H7. Iraqi J. Vet. Med. 2023;47(1):86-92

A B S T R A C T

E. coli O157:H7, is one of the main causes of diarrhea and the most prevalent bacterial infection that causes serious illnesses. This research was conducted to investigate the effect of *Capparis spinosa* fruit ultrasonic extract against resistant *E. coli* O157:H7 was isolated from stools of calves that were suffering from diarrhea; the bacteria were identified by the Vitek 2 system and a latex agglutination test. *C. spinosa* was extracted by ultrasonic waves water bath. The phytochemicals were carried out on *C. spinosa* fruit extract to detect the secondary metabolites. The MIC of the extract was calculated with concentrations of 400, 800, 1600, 3200, 6400, 12800 and 25600 51200 µg/mL by microdilution method (checkerboard). While using a field-emission scanning electron microscope to observe the morphological alterations in *E. coli* O157:H7 sample. The findings of this study revealed that the extract contains some biologically active compounds like alkaloids, flavonoids, steroids, glycosides, tannins, cumarines, saponins, quinones, and amino acids. That extract of *C. spinosa* had a MIC of 6400 µg/mL and had a perfect action against *E. coli*. O157:H7 by forming vacuoles within the cells and that internal content had seeped out as pore formation. This finding could potentially provide an explanation for the traditional utilization of this plant material as an antibacterial agent.

Keywords: *Capparis spinosa,* fruit, methanolic, agglutination test, *E. coli* O157:H7

INTRODUCTION

Escherichia coli (*E. coli*) is commonly found in the intestinal flora of both animals and humans. While many strains are harmless, certain serotypes, such as the enterohemorrhagic *E. coli* O157, are pathogenic and can cause illnesses ranging from moderate to fatal (1, 2). *E. coli* O157:H7 is a predominant bacterial infection responsible for severe conditions like hemolytic uremic syndrome, kidney failure, abdominal pain, and bloody diarrhea (3). Cattle serve as the primary reservoir for *E. coli* O157:H7, making it a significant global foodborne zoonotic pathogen (4, 5). The prevalence of *E. coli* O157:H7 in dairy components can be attributed to inadequate sanitation, insufficient post-milking teat dipping, improper labor practices, and inconsistent milking schedules for cows of different ages. Moreover, its presence is often noted on dairy farms that lack proper therapeutic measures (6). Factors such as lactation stage, weather conditions, feed quality, and the bacteria's environmental persistence contribute to the risk of *E. coli* O157:H7 infection in animals (7). Infectious calf diarrhea is a major concern for newborn calves, impacting their growth, morbidity, mortality, and causing significant economic losses globally (7, 8). *E. coli* O157:H7 belongs to the broader category of Shiga toxin-producing *E. coli*, which can produce Shiga toxin types 1, 2, or both, along with their variants (7, 9).

Capparis spinosa L. (Caper) is a spiny annual shrub from the Capparidaceae family. Its ripe fruits, known for their spicy and pungent flavor, have been traditionally used to address gastrointestinal issues, urinary infections, and cardiovascular discomforts (10). *C. spinosa* thrives in

regions like Italy, South Europe, Madagascar, North and East Africa, Greece, Southwest and Central Asia, Australia, Oceania, Iraq, and Iran (11, 12). Chemical studies have identified numerous beneficial compounds in *C. spinosa*, including glucocapperin, lipids, flavonoids, alkaloids, saponins, tannins, lignin, and hydroxy-3-oxo-ionolglucosides (13-15). Traditionally, various parts of the plant have been used to treat gastrointestinal issues, skin ailments, earaches, and liver and kidney problems (16). Recent research highlights its antibacterial and antifungal properties (17). Notably, *C. spinosa* exhibits stronger antimicrobial activity against Gram-negative bacteria like *E. coli*, *Salmonella typhi*, and *Shigella dysenteriae* than against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus panis* (18). Aerial parts of *C. spinosa* are believed to contain antibacterial compounds, with extracts showing efficacy against multiple bacterial strains, especially antibiotic-resistant ones such as *E. coli* (12). This study aims to investigate the efficacy of *C. spinosa* fruit ultrasonic extract against *E. coli* O157:H7, given its role as a primary cause of diarrhea and economic losses, especially in calves.

MATERIALS AND METHODS

E. coli O157:H7

The *E. coli* O157:H7 strain was isolated from stool samples of calves exhibiting diarrhea. The bacterial strain was identified as *E. coli* O157:H7 using the Vitek 2 system, which employs a fluorogenic methodology for the swift and precise identification of both gram-negative and gram-positive bacteria. Additionally, a latex agglutination test was conducted on the bacterial isolate as per the manufacturer's instructions (Wellcolex *E. coli* O157:H7, Remel).

Ultrasound-Assisted Extraction of *C. spinosa* Fruit

One hundred mL of 99.8% methanol (Fluka, Switzerland) was combined with 10 g of powdered *C. spinosa* fruit in a flask. The *C. spinosa* fruit, sourced from a local Iraqi market, was authenticated by the Iraqi National Herbarium, Directorate of Seed Testing and Certification, Iraqi Ministry of Agriculture, in Abu-Ghraib, Baghdad, Iraq (Certification No. 2861 on 11/11/2021). The mixture underwent ultrasonic treatment in a water bath (Sinobakr, China) for an hour at 60 kHz frequency, in the dark, and at room temperature. This procedure was repeated ten times under identical conditions. Subsequently, the mixture was filtered using filter paper (Sartorius, Germany). The filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland). The resulting extract was stored at -18 °C until further use (21).

Preliminary Qualitative Phytochemical Screening

Standard methods were employed to detect secondary metabolites in the *C. spinosa* fruit extract as follows:

Alkaloids Detection (Mayer Test)

Mayer's reagent was added to 1 cm^3 of the extract. The formation of a white or pinkish-white precipitate indicates the presence of alkaloids (23).

Flavonoids Detection (Shinoda's Test)

A few drops of concentrated hydrochloric acid were added to 1 cm3 of the extract, then a small piece of magnesium tape or its powder was added, which would have a red color indicating the presence of flavonoids (24).

Steroids Detection (Salkowski's Test)

To 1 cm^3 of the extract, a few drops of concentrated sulfuric acid (H_2SO_4) were added slowly and over the walls. A bright red color indicates the presence of steroids (25).

Glycoside Detection (Keller-Kilini Test)

1 cm 3 of glacial acetic acid was added to 1 cm 3 of the extract, then cooled it in a water bath and two drops of ferric chloride solution were added to it, then the contents of the test tube were poured into another tube containing 2 cm^3 of concentrated sulfuric acid. A red ring indicates the presence of sugars (glycosides) (26).

Phenolic Compounds and Tannins Detection (Ferric Chloride Test)

A few drops of 0.1% ferric chloride were added to 1 cm^3 of the extract. A green or blackish-green color indicates the presence of tannins or phenolic compounds, while a brown color suggests a false positive for tannins (27).

Coumarins Detection (Sodium Hydroxide Test)

One cm^3 of the extract was combined with 1 cm^3 of distilled water in a test tube. The tube was sealed with filter paper soaked in 7% sodium hydroxide and placed in a boiling water bath. A phosphorescent greenish-yellow color indicates the presence of coumarins (28).

Saponins Detection (Mercuric Chloride's Test)

A mixture of 1 cm^3 of extract and 1 cm^3 of $HgCl_2$ at 1% was created. The emergence of a white precipitate suggests the presence of saponins (29).

Quinones Detection (Potassium Chloride's Test)

The extract was sprayed with an alcoholic potassium hydroxide solution, prepared by dissolving a trace amount of potassium hydroxide in alcohol. A color transition from red to blue indicates the presence of quinones (30).

Amino Acids and Protein Detection (Ninhydrine's Test)

Drops of 0.2% ninhydrine reagent were added to 1 cm^3 of the extract, which was subsequently heated in a water

bath. A blue hue signifies the presence of free amino acids (31).

Determination of Minimum Inhibitory Concentration (MIC) of *C. spinosa* Fruit Extract

A stock solution of *C. spinosa* fruit extract was formulated in Mueller-Hinton broth (MHB, HiMedia, India). Concentrations of 400, 800, 1600, 3200, 6400, 12800, 25600, and 51200 µg/mL of the extract were prepared in a 96-well microtiter plate with a U-shaped bottom. Each well received an inoculation of 100 µL of 10^6 CFU/mL *E. coli* O157:H7 and was incubated at 37 °C for 22 h (30). For the negative control, 200 µL of MHB was added to blank wells, which were devoid of microorganisms. To colorimetrically identify bacterial growth, 20 µL of 2,3,5-triphenyltetrazolium chloride (TTC) indicator (0.125% w/v) was introduced. This solution, prepared at a concentration of 0.125% w/v in sterile deionized water, was added to each test well and re-incubated for an additional 2 h (31).

Scanning Electron Microscope

The methodology, adapted from Bajpai et al. (32), was utilized to examine morphological changes using a field-emission scanning electron microscope (Fe-SEM, Alkora, FEI Co., Inspect S50, Holland). Two tubes were prepared, each containing 100 µL of bacterial suspensions (1×10^8 CFU/mL) inoculated onto MHB. One tube was supplemented with the MIC value of *C. spinosa* fruit extract, while the other, serving as the positive control, contained only the bacterial suspension. Both tubes were incubated at 37 °C for 4 h. Subsequently, 600 µL of each aliquot was dispensed onto glass coverslips (1×1 cm). Post-incubation, the coverslips were rinsed thrice with 0.1 M phosphate buffer saline. This was followed by the addition of 1 mL each of 3% glutaraldehyde (Sigma, USA) and 2% paraformaldehyde (Sigma, USA) in 0.1 M potassium phosphate buffer. After three buffer washes, the samples underwent a graded dehydration process using increasing ethanol concentrations (50%, 60%, 70%, 80%, 90%, and 100%, Merck, UK). The dehydrated samples were then stored in a silica desiccator (Labbox, UK) for 72 h to ensure thorough drying. Prior to SEM analysis, the samples underwent a 2-minute gold sputtering process in an ion coater. The samples were then examined under the Fe-SEM. The primary objective of using the Fe-SEM was to elucidate the mechanism of action of the *C. spinosa* fruit extract against *E. coli* O157:H7. This included observing alterations in bacterial size, shape, and overall morphology post-extract treatment.

Results and Discussion

Phytochemical Analysis

The phytochemical tests of *C. spinosa* fruit that extracted by methanol showed that several compounds were present in fruit. results showed, the fruit containing alkaloids, flavonoids, steroids, glycosides, carbohydrates, tannins, phenolic compounds, saponins and absent of terpin (Table 1).

Table 1. Phytoconstituent of methanolic extract *C. spinosa* fruit

Compounds	Results
Alkaloids	++++
Amino acids	+++
Cumarines	Trace
Flavonoids	+
Glycoside	+++
Quinones	+
Saponins	++
Steroids	Full
Tannins	+

This result of phytochemical screening agreed with those obtained by (34). Large amounts of phenolics and flavonoids, particularly quercetin-3-O-rutinoside (rutin), a glycoside of the flavonoid quercetin, were found in plant extracts after in vitro examination. According to the already published literature, rutin was the main flavonoid (35). Phytochemical analysis of caper extracts by Tagnaout et al. (36) revealed the presence of numerous bioactive chemicals from a variety of chemical families, including, alkaloids, flavonoids, fatty acids, esters, aldehydes and glucosinolates.

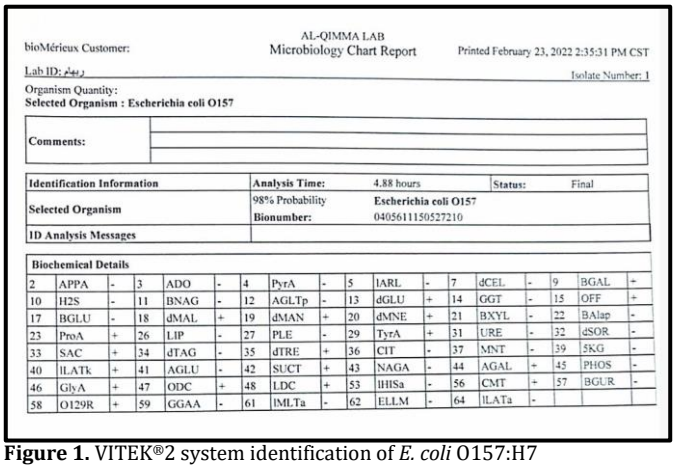
Recent studies indicated phenols and flavonoids as significant bioactive natural compounds obtained from medicinal plants (37). The findings were consistent with those reported in earlier studies by Wojdyo et al. (39), showing the plant's therapeutic and vital importance as a result of the compounds' potency in physiologic processes and defense mechanisms against bacterial and fungal pathogens that infect living organisms. The results are also in agreement with Hamad et al (40) that reported that could be seen several organic compounds, including tannins, terpins, alkaloids, flavonoids, resins, glucosides quinones, steroids, saponin sides, and phenolic compounds were presented in all the parts of the *C. spinosa* plant Quinones are derivatives of phenols, which are present in all parts of the *C. spinosa* plant. They were ubiquitous in nature and were characteristically highly reactive (42).

The existence of active compounds including flavonoids, alkaloids, glycosides, phlobatannins, and saponins in natural fruit demonstrated their great significance and reveals their medicinal importance that was once used and is currently used. The plant is powerful against numerous bacterial infections since it was a great antioxidant (43) functional groups of tannins in the crude *C. spinosa* extract could form hydrogen bonds with proteins and carbohydrates in the bacterial cells. If this happens, they may inhibit essential enzymes for bacterial growth (35).

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

The isolated bacteria have achieved an excellent identification level with a probability of 98% based on the manufacturers' technical datasheet (Figure 1). The findings

showed that *E. coli* O157:H7 was identified with excellent ID message confidence levels (probability percentages between 93 and 98%). For the majority of infectious disease pathogens, bacterial identification done automatically in a clinical laboratory offers a quick and accurate diagnosis with a very high level of identification accuracy (45).



bioMérieux Customer: AL-QIMMA LAB Microbiology Chart Report Printed February 23, 2022 2:35:31 PM CST

Lab ID: [illegible] Isolate Number: 1

Organism Quantity:

Selected Organism : Escherichia coli O157

Comments:

Identification Information	Analysis Time:	4.88 hours	Status:	Final
Selected Organism	98% Probability	Escherichia coli O157		
	Bionumber:	0405611150527210		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 1. VITEK®2 system identification of *E. coli* O157:H7

Serotyping Test (Wellcolex *E. coli* O157:H7, Remel) Latex Agglutination Test

The O157 and H7 antigens of *E. coli* colonies were identified using the wellcolex *E. coli* O157:H7, remel. O157:H7-reactive isolates were sub cultured overnight on blood agar to detect flagellar antigen (H7). Red color agglutination revealed a positive result for (O antigen), when compared to the obvious red color of the control. whereas blue color agglutination suggested a positive result for (H antigen), (Figure 2).

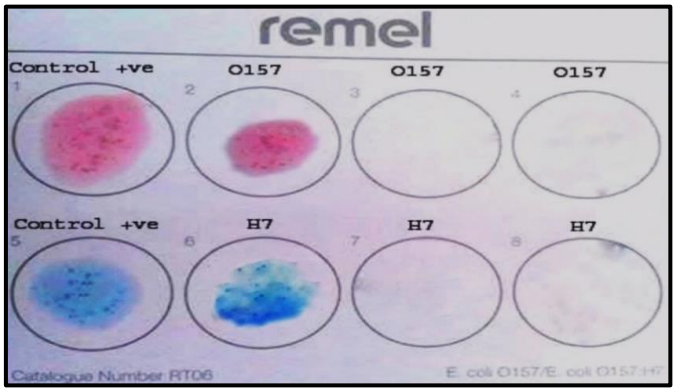


Figure 2. Positive results of O157 and H7 antigens with latex agglutination test

This result was in agreement with Al-Taae (46), who used the latex agglutination test to identify *E. coli* O157:H7 and showed that this test was specific and sensitive in recognizing *E. coli* O157:H7 isolates. It was also easy and rapid to perform and interpret. This test was used by Khalaf (47) as a quick way to identify *E. coli* O157:H7 and separate it from other pathogenic *E. coli* serotypes. Fesseha et al. (48) also isolated *E. coli* O157:H7 from calves' fecal samples and confirmed the isolates using the latex agglutination test.

MIC

Based on the test of latex agglutination that confirmed the identity of *E. coli* O157:H7 isolate was challenged against different concentrations of *C. spinosa* fruit extract to determine the minimum inhibitory concentration (MIC). The results showed that the concentration 6400 µg/ml of *C. spinosa* fruit extract was active against *E. coli* O157:H7 isolate. The tested MIC in micro-dilution assay is shown in Figure 3. Similarly. Based on visual readings Veiga et al. (31) carried out by seeing whether or when the red color that results from the reductions of TTC (colorless) to formazan (red) develops. The MIC was determined using the lowest concentration and without any color development, confirming the results shown in the experiment. Many methods were used to determine the MIC, but the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute both consider the microdilution assay to be the most widely used and accredited method for determining the MIC (49). This result was in agreement with Abu-Shama (50) which reported the MIC of *C. spinosa* fruit extract against *E. coli* O157:H7 640 mg/100 mL.

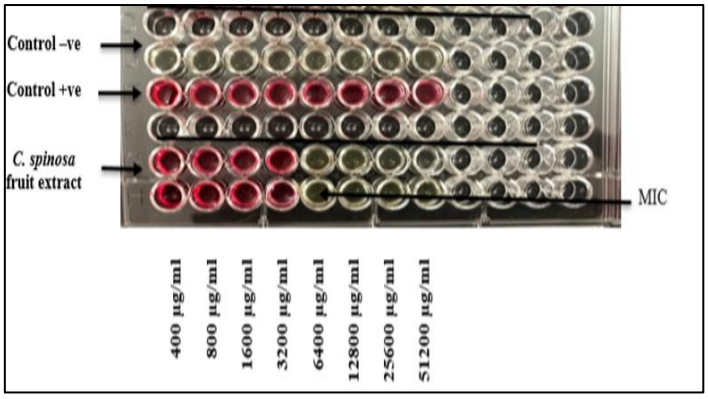


Figure 3. Checkerboard assay for minimum inhibitory concentration of *C. spinosa* fruit extract against *E. coli* O157:H7 isolate (white: no growth, red: growth

FE-SEM

To describe the size, shape, and morphology of the bacteria after treatment with antibacterial substances, field- emission scanning electron microscopy (Fe-SEM) analysis is used. Fe-SEM analysis was used to detect changes in the surface morphology and integrity of the cell membrane of *E. coli* O157:H7 cells after exposure to several antibacterial agents. The results of Fe-SEM showed the bacterial cells of the positive control group appeared to be of normal size and had, a typical rod-like shape with intact and smooth cell surfaces. We noticed through an image of bacteria (*E. coli* O157:H7), which was considered without treatment, that the (Figure 4) shows the growth of the

bacteria in a dense manner in the form of active growth clusters. The length of these micro clusters (1.0 micrometers) was considered a positive control to study the effect of antibacterial additives. The live bacterial system is considered free of therapeutic additives from *C. spinosa* fruit extract. While the cell walls of the bacteria that were treated with 1× MIC (6400 µg/mL) of *C. spinosa* fruit extract showed that there were vacuoles within the cells and that internal contents had seeped out as pore formation and *E. coli* O157:H7 cell lysis (Figure 5).

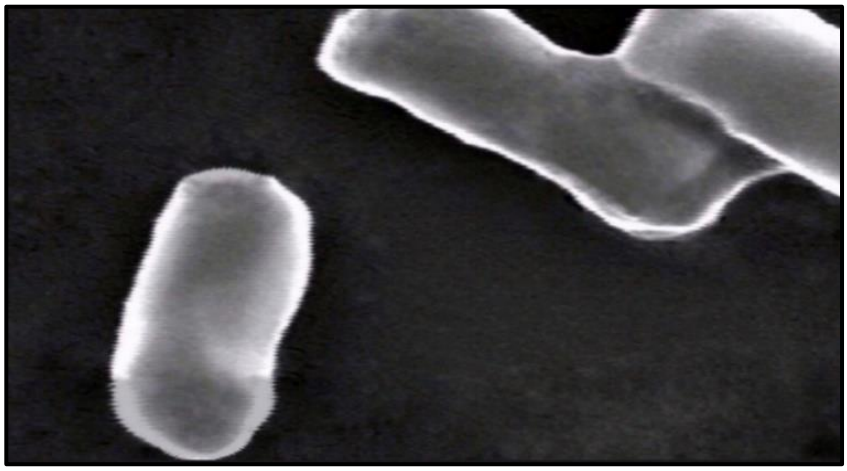


Figure 4. Field emission scanning electron microscopy photographs in micrometers of positive control E. coli O157: H7 shows normal sizes, a typical rod-like shape with smooth cell surfaces of bacterial cells, mag: 50000×, pressure: 1.10e-2pa, Inspect F50 FE-SEM: 1 µm

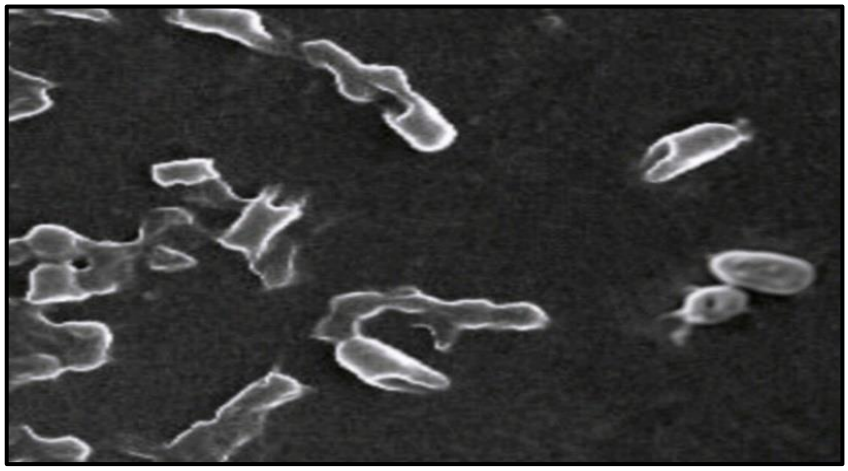


Figure 5. Field emission scanning electron microscopy photographs of reveal the antibacterial effect of *C. spinosa* fruit extract on *E. coli* O157: H7 shows vacuoles within the cells and that internal contents are seeped out as pore formation, mag: 7000×, pressure: 8.36e-3pa, Inspect F50 FE-SEM: 10 µm

To explore how an agent affects the shape and size of the bacteria, Fe-SEM photos needed to be recorded (51). Hamad et al. (40) indicated that the activity of crude *C. spinosa* extracts was greatest at its maximum concentration (400 mg/mL). This activity might result from its chelating nature or its ability to dissolve the lipid layer of bacterial cell walls, causing the destruction of bacterial liquids and likely leading to the formation of hydrogen bonds with hydroxyl and nitrogen groups of the crude *C. spinosa* extracts, with water molecules being found in the bacterial cells (52). The antibacterial effect of *C. spinosa* fruit extract on *E. coli* O157:H7 may be attributed to the compound present in extract that helps in the formation vacuoles within the cells and that internal contents had seeped out as pore formation.

ACKNOWLEDGEMENTS

N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Aljeboury GH. A comparative study of amoxicillin sensitivity against *Escherichia coli* isolates isolated from urinary tract infections. Iraqi Sci. 2017;417-426.
2. Al-Khyat FA. Enterohaemorrhagic *E. coli* O157 in locally produced soft cheese. Iraqi J. Vet. Med. 2008;32(1):89-99.

3. AL-Imam MJ, Flayyih MT. Molecular study of regulatory gene (*Ler*) in Enteropathogenic *Escherichia coli* (EPEC) of diarrheagenic patients. Iraqi J. Sci. 2020;61(10):2486-2493.
4. Al-Taii DH, Yousif AA. Effects of *E. coli* O157:H7 experimental infections on rabbits. Iraqi J. Vet. Med. 2019;43(1):34-42.
5. Yaseen SM, Saleh AM, Al-Zubaidy RS. Contamination of the local produced broilers carcasses with *Escherichia coli* O157:H7 and its effect in public health in Diyala province. Iraqi J. Vet. Medicine. 2017;41(2):113-117.
6. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th ed. Edinburgh: Elsevier; 2016.
7. Saeedi P, Yazdanparast M, Behzadi E, Salmanian AH, Mousavi SL, Nazarian S, et al. A review on strategies for decreasing *E. coli* O157:H7 risk in animals. Microb. patho. 2017;103:186-195.
8. Najim NH. The synergistic bactericidal effects of bacteriocin and pressurization against *E. coli* O157:H7 in raw milk. Iraqi J. Vet. Med. 2014;38(1):15-23.
9. Al–Dawmy FA, Yousif AA. Prevalence of *E. coli* O157:H7 in intestinal and urinary tract infection in children. Int. J. Adv. Res. 2013;1(8):111-120.
10. Al-Tamimi A, Khatib M, Pieraccini G, Mulinacci N. Quaternary ammonium compounds in roots and leaves of *Capparis spinosa* L. from Saudi Arabia and Italy: investigation by HPLC-MS and ^{1}H NMR. Nat. Prod. Res. 2019;33(9):1322-1328.
11. Zarei M, Seyedi N, Maghsoudi S, Nejad MS, Sheibani H. Green synthesis of Ag nanoparticles on the modified graphene oxide using *Capparis spinosa* fruit extract for catalytic reduction of organic dyes. Inorganic Chem. Commun. 2021;123:108327.
12. AL-Azawi AH, Ghaima KK, Salih HH. Phytochemical, antibacterial and antioxidant activities of *Capparis spinosa* L. Cultivated in Iraq. Bio. Res. 2018;15(3):2611-2618.
13. Al-Khafagi MF, Mohammed DY. Study antibacterial activity of crude *Capparis spinosa* L. extracts against *Helicobacter pylori* infection and determine their bioactive compounds. Iraqi J. Sci. 2023;64(2):503-512.
14. Al Janabi NM, Al Badri SR. Effect of temperature and pH on antioxidant effectiveness of *Capparis spinosa* leaves. Iraqi J. Biotech. 2019;18(2):150-159.
15. Shahrajabian MH, Sun W, Cheng Q. Plant of the Millennium, Caper (*Capparis spinosa* L.), chemical composition and medicinal uses. Bull. Nat. Res. Cen. 2021;45:131.
16. Mollica A, Zengin G, Locatelli M, Stefanucci A, Mocan A, Macedonio G, Carradori S, et al. Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: in vivo and in vitro evaluation of its nutraceutical potential. J. Funct. Food. 2017;35:32-42.
17. Benzidane N, Aichour R, Guettaf S, Laadel N, Khennouf S, Baghiani A, et al. Chemical investigation, the antibacterial and antifungal activity of different parts of *Capparis spinosa* extracts. J. Drug Deliv. Therap. 2020;10(5):118-125.
18. Mazarei F, Jooyandeh H, Noshad M, Hojjati M. Polysaccharide of caper (*Capparis spinosa* L.) Leaf: extraction optimization, antioxidant potential and antimicrobial activity. International J. Bio. Macro. 2017; 95:224-2231.
19. Arean AG, Ali TH, Muraih JK. Extracted chemical compounds from *Capparis spinosa* leaves and their antibacterial activity on pathogenic bacteria. J. Pharma. Sci. Res. 2019;11(2):603-608.
20. Abdulridha RN, Saliem AH. Antidiarrheal effect of *Capparis spinosa* fruits extract. Egypt. J. Hosp. Med. 2023; 91(1):3862-3869.
21. Ghorbani M, Aboonajmi M, Ghorbani JM, Arabhosseini A. Effect of ultrasound extraction conditions on yield and antioxidant properties of the fennel seed (*Foeniculum vulgare*) extract. Iran. J. Food. 2017;14(67):63-73.
22. Velavan S. Phytochemical techniques-a review. World J. Sci. Res. 2015;1(2):80-91.
23. Paech K, Tracey MV. Modern Methods of Plant Analysis/Moderne Methoden der Pflanzenanalyse. Spr. Sci. and Bus. Med. 2013; 2.
24. Egbuna C, Ifemeje JC, Maduako MC, Tijjani H, Udedi SC, Nwaka AC, Ifemeje MO. Phytochemical test methods: qualitative, quantitative and proximate analysis. In. Phyto. Chem. 2018; 381-426. Apple Academic Press.
25. Harborne JB. Phytochemical Methods. New Delhi: Springer (India) Pvt. Ltd; 2005.
26. Evans WC, Trease GE, editors. Pharmacognosy. 15th ed. London: WB Sanders Publishing Company; 2002. p. 277-280.
27. Harborne JB, editor. The Flavonoids: Advances in Research since 1980. London: Chapman and Hall; 1994.
28. Kapali J. Biological studies of some selected medicinal plants from Kathmandu Valley and isolation of chemical compounds [dissertation]. Nepal: Tribhuvan University; 2021.
29. Bla BK, Dagnogo O, Kipré RG, Ballé OG, Trébissou JD, Djaman JA. Antiplasmodial activity of *Anthocleista djalonensis* leaves extracts against clinical isolates of *Plasmodium falciparum* and multidrug resistant K1 strains. World J. Adv. Res Rev. 2020;8(3):130-138.
30. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. CLSI supplement VET08. Wayne (PA): Clinical and Laboratory Standards Institute; 2018.
31. Veiga A, Maria da Graça TT, Rossa LS, Mengarda M, Stofella NC, Oliveira LJ, et al. Colorimetric microdilution assay: Validation of a standard method for determination of MIC, $IC_{50\%}$, and $IC_{90\%}$ of antimicrobial compounds. J. Micro. Meth. 2019;162:50-61.
32. Bajpai VK, Sharma A, Baek KH. Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. Food Cont. 2013; 32(2):582-590.
33. Reed SJ. Electron microprobe analysis and scanning electron microscopy in geology. Cambridge: Cambridge University Press; 2005.
34. Siracusa L, Kulisic-Bilusic T, Politeo O, Krause I, Dejanovic B, Ruberto G. Phenolic composition and antioxidant activity of aqueous infusions from *Capparis spinosa* L. and *Crithmum maritimum* L. before and after submission to a two-step in vitro digestion model. J Agri. Food Chem. 2011;59(23):12453-12459.
35. Tlili N, Khaldi A, Triki S, Munné-Bosch S. Phenolic compounds and vitamin antioxidants of caper (*Capparis spinosa*). Plant Food Hum. Nut. 2010;65:260-265.
36. Tagnaout I, Zerkani H, Mahjoubi M, Bourakhouadar M, Alistiqsa F, Bouzoubaa A, Zair T. Phytochemical study, antibacterial and antioxidant activities of extracts of *Capparis spinosa* L. Int. J. Pharma. Phyto. Res. 2016; 8(12):1993-2006.
37. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Med. 2018; 5(3):93.
38. Shaito A, Thuan DT, Phu HT, Nguyen TH, Hasan H, Halabi S, Abdelhady S, Nasrallah GK, Eid AH, Pintus G. Herbal medicine for cardiovascular diseases: efficacy, mechanisms, and safety. Fron. Pharma. 2020;11:422.
39. Wojdyło A, Nowicka P, Grimalt M, Legua P, Almansa MS, Amorós A, Carbonell-Barrachina ÁA, Hernández F. Polyphenol compounds and biological activity of caper (*Capparis spinosa* L.) flowers buds. Plants. 2019; 8(12):539.
40. Hamad LR, Hussain AB, Hassan MH. A Pharmacological effect of *Copparis spinosa* extracts on pathogenic *Escherichia coli*. Int J Pharm Res. 2020;12(2):0975-2366.
41. Hameed AT, Zaidan DH, Dawd SM. The phytochemical constituent of *Capparis spinosa* L. and phenolic activity on pathogenic bacteria and blood parameters. Sys Rev Pharm. 2021;12(1):1193-1198.
42. Oudah SK, Al-Salih RM, Gusar SH. Study the role of polyphenolic extract of *Capparis spinosa* L. leaves as a hypoglycemic agent. Int. J. Sci. Eng. Res. 2014;5(5):1561-1575.
43. Yahia Y, Benabderrahim MA, Tlili N, Hannachi H, Ayadi L, Elfalleh W. Comparison of three extraction protocols for the characterization of caper (*Capparis spinosa* L.) leaf extracts: Evaluation of phenolic acids and flavonoids by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS) and the antioxidant activity. Anal Let. 2020;53(9):1366-1377.

44. Hussein NM, Atea AM, Humide AO, Abdullah QK, Hardan SM. Isolation and diagnosis of bacteria causing urinary tract infection in children. Sys Rev Pharm. 2020;11(1):76-79
45. Paim TG, Cantarelli VV, d'Azevedo PA. Performance of the Vitek 2 system software version 5.03 in the bacterial identification and antimicrobial susceptibility test: evaluation study of clinical and reference strains of Gram-positive cocci. Rev. Soci. Bras. Med. Trop. 2014; 47:377-381.
46. Al-Taae DH. Isolation and identification of *E. coli* O157:H7 from diarrheal children and animals and study the biochemical and pathological changes in rabbits [Thesis]. Baghdad: University of Baghdad; 2015.
47. Khalaf FA. Prevalence of *E. coli* O157:H7 in Karballa province in human and animals and in vivo study of rabbits antisera as a diagnostic tool [Thesis]. Baghdad: University of Baghdad; 2014.
48. Fesseha H, Mathewos M, Aliye S, Mekonnen E. Isolation and antibiogram of *Escherichia coli* O157:H7 from diarrhoeic calves in urban and peri-urban dairy farms of Hawassa town. Vet Med Sci. 2022; 8(2):864-76.
49. Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R, Banat IM. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotech. Letters. 2016; 38:1015-1019.
50. Abu-Shama HS. Effect of Caper (*Capparis spinosa*) extracts as a natural antimicrobial agent. J. Food Dairy Sci. 2019;10(7): 209-216.
51. Bazin D, Bouderlique E, Daudon M, Frochot V, Haymann JP, Letavernier E, et al. Scanning electron microscopy—a powerful imaging technique for the clinician. Comptes Rendus. Chimie. 2022;25(S1):37-60.
52. Mahboubi M, Mahboubi A. Antimicrobial activity of as its usages in traditional medicine. Herb. Polonica. 2014;60(1):39-48.

تأثير المستخلص بالموجات فوق الصوتية لفاكهة الشفلح ضد الإيشيريشيا القولونية O157:H7

ريهام نجم عبد الرضا، علي حسين سليم

فرع الفسلجة والكيمياء الحياتية والادوية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

الإيشيريشيا القولونية O157: H7 أحد الأسباب الرئيسية للإسهال حيث تعتبر العدوى البكتيرية الأكثر انتشارًا التي تسبب أمراضًا خطيرة، وقد تم إجراء هذا البحث لمعرفة آلية عمل مستخلص فاكهة الشفلح بالموجات فوق الصوتية ضد الإيشيريشيا القولونية المقاومة O157: H7 (المعزولة من براز العجول التي تعاني من الإسهال)، تم التعرف على البكتيريا بواسطة نظام Vitek 2 واختبار تراص اللاتكس الذي يعتمد على تفاعل الأجسام المضادة للمستضد، وتم استخلاص ثمرة الشفلح بالموجات فوق الصوتية. تم اجراء التحليل الكيميائي النباتي للكشف عن المستقلبات الثانوية. تم حساب التركيز المثبط الأدنى للمستخلص بتراكيز(٤٠٠، ٨٠٠، ١٦٠٠، ٣٢٠٠، ٦٤٠٠، ١٢٨٠٠، ٢٥٦٠٠ و ٥١٢٠٠ ميكروغرام/ مل) بطريقة التخفيف الدقيق. استخدم المجهر الإلكتروني الماسح Fe-SEM لمراقبة التغيرات الشكلية في عينة الإيشيريشيا القولونية O157: H7. كشفت نتائج هذا البحث أن المستخلص يحتوي على العديد من المركبات النشطة بيولوجيا مثل القلويات والفلافونويد والستيرويدات والجليكوسيد والعفص والكومارين والصابونين والكينون والأحماض الأمينية. هذا المستخلص من ثمرة الشفلح سجل تركيز تثبيط ادنى ضد الإيشيريشيا القولونية (٦٤٠٠ ميكروغرام/ مل) وله آلية عمل مثالية ضد الإيشيريشيا القولونية O157: H7 عن طريق تكوين فجوات داخل الخلايا وقد تسرب هذا المحتوى الداخلي على شكل تشكيل مسام. يمكن أن توفر هذه النتيجة تفسيرًا للاستخدام التقليدي لهذه المادة النباتية كعامل مضاد للبكتيريا.

الكلمات المفتاحية: الشفلح، فاكهة، ميثانول، اختبار التلازن، الإيشيريشيا القولونية O157:H7